RED KING CRAB HATCHERY CULTURE AND ECOLOGICAL REQUIREMENTS:

APPLICATIONS FOR STOCK ENHANCEMENT

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RED KING CRAB HATCHERY CULTURE AND ECOLOGICAL REQUIREMENTS: APPLICATIONS FOR STOCK ENHANCEMENT

A

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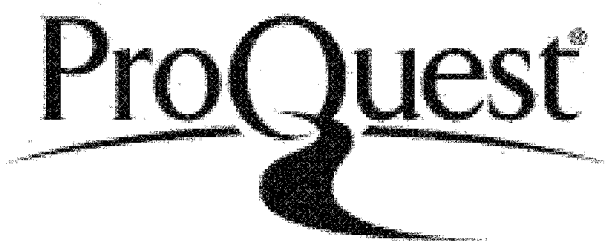

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ABSTRACT

The ecologically and commercially important red king crab (*Paralithodes camtschaticus*) is depleted throughout much of the North Pacific and thought to be recruitment limited, making it an appropriate candidate for stock enhancement efforts. This research addresses bottlenecks associated with hatchery production and lays the groundwork for developing release strategies. I investigated effects of diet, stocking density, and size grading on survival, growth, and shell coloration of recently-settled juvenile red king crabs in large-scale hatchery experiments. I also conducted laboratory experiments with fish predators to determine if red king crab predator responses could be enhanced with experience. Finally, I tethered hatchery-cultured red king crabs of two sizes in the field for 24 h trials and used underwater video cameras to identify predators and predation susceptibility. In hatchery experiments, dietary astaxanthin supplementation improved survival and shell coloration suggesting that red king crab coloration is plastic and that astaxanthin may provide nutritional or immune system benefits. Size grading strongly influenced survival and growth in the hatchery. Generally, small crabs had higher survival than large and ungraded crabs, but large and ungraded crabs had higher growth, likely from cannibalism. In laboratory experiments, halibut exposure enhanced red king crab crypsis and survival suggesting that cryptic behavior is plastic and may be enhanced with experience. In the field experiment, I identified specific predators of recently-settled red king crabs in a nearshore habitat and showed that survival did not vary with body size or deployment month during the first juvenile instar stages. My research provides an important step for developing a responsible red

king crab stock enhancement program by demonstrating that hatchery production can be improved with specific advances in rearing technology, hatchery-cultured red king crabs are morphologically and behaviorally plastic, hatchery-cultured red king crabs tethered in the field show no obvious behavioral deficiencies that may exacerbate predation, and that differences in predation susceptibility during the first juvenile instar stages are subtle and may be ecologically inconsequential for post-release survival. As bottlenecks in hatchery production and survival of released juveniles are overcome, stock enhancement will become increasingly feasible for red king crabs in Alaska.

DEDICATION

to Beaté and Grace

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GENERAL INTRODUCTION

The growing global demand for seafood has increased pressure on wild fish stocks (Pauly et al., 1998). Approximately 80% of world fish stocks are currently fully exploited or overexploited with little signs of recovery (FAO, 2010). Aquaculture and stock enhancement have been suggested as possible approaches to meet increased global demand by enhancing or restoring depressed fisheries and are currently in progress for many species worldwide (Leber et al., 2004; Bell et al., 2005). Stock enhancement is a controversial fisheries management tool, mainly because of concerns with negative impacts on wild stocks either by competitive displacement, loss of genetic diversity, increased fishing pressure, or relaxed management measures (Bell et al., 2008; Kitada et al., 2009). Additionally, few stock enhancement programs are deemed a success, largely due to the inability to quantify post-release survival or effects of large-scale releases on wild populations (Blankenship and Leber, 1995; Lorenzen et al., 2010). A conceptual framework known as the "Responsible Approach" was developed to help design, evaluate, and manage marine stock enhancement programs (Blankenship and Leber, 1995; Lorenzen et al., 2010) and recent technological advances in aquaculture, genetics, tagging, and fishery modeling have enabled fishery scientists to begin to quantitatively evaluate effects of stocking cultured fish from a broad ecological and management perspective (Bell et al., 2008).

Hatchery production technology has made great strides and has enabled large-scale production of finfish and crustaceans. Worldwide aquaculture is rapidly expanding from a 3.9% contribution to global seafood production in 1970 to a 36.9% contribution in

2008 with an average annual growth rate of 8.3% (FAO, 2010). Specifically, there has been much advancement in crab culturing technology. Warm water species such as mud crab (*Scylla Paramamosain*), Chinese mitten crab (*Eriocheir japonica sinensis*), and blue crab (*Callinectes sapidus*) are cultured on a massive scale (Christensen et al., 2004; Zmora et al., 2005; Cheng et al., 2008) and there has been progress in large-scale production of cold water crab species. Japanese scientists began cultivating king crabs in the 1960s (Kurata, 1960a, 1960b, 1961), and considerable advancements in king crab culture have been made in Japan (Nakanishi and Naryu, 1981; Nakanishi, 1987, 1988; Stevens 2006a), Argentina (Lovrich and Tapella, 2006), Russia (Epelbaum et al., 2006; Kovatcheva et al., 2006), and the United States (Stevens et al., 2008; Daly et al., 2009; Swingle et al., in review).

King crabs are some of the most commercially valuable crustaceans in the world. Historically, red king crab (*Paralithodes camtschaticus*) was one of the most important fisheries in Alaska, USA; however, populations have fluctuated over the past 30 years (Stevens et al., 2001). Five of the nine historical red king crab fisheries in Alaska including Kodiak, Cook Inlet, Prince William Sound, the Aleutians, and the Alaska Peninsula were closed in the 1980s, while the Pribilof Island red king crab fishery was closed in 1999. Despite these closures, abundances have not recovered, and fisheries remain closed today. Only the Bristol Bay and Norton Sound fisheries are consistently open. The Southeast Alaska fishery is intermittently open and closed due to fluctuating estimates of stock abundance (Stratman et al., 2011). The cause of population fluctuations and diminished stock size is unclear; however, recruitment limitation (Blau,

1986), groundfish predation (Bechtol and Kruse, 2009), egg predation (Kuris et al., 1991), disease, overfishing (Orensanz et al., 1998), and climate change (Zheng and Kruse, 2006) have been proposed to explain the lack of recovery.

Traditional management techniques have not helped Alaskan red king crab stocks recover, causing fisherman, scientists, and managers to seek alternative solutions. King crabs are suitable candidates for stock enhancement because of their high commercial value and the fact that recruitment limitation has been proposed to explain their lack of recovery in the absence of fishing (Blau, 1986). The Alaska King Crab Research and Rehabilitation and Biology (AKCRRAB) program was created in 2006 to assess the feasibility of stock enhancement for king crabs in Alaska, with the ultimate goal of rehabilitating depressed populations by increasing spawning stock abundance through the release of cultured juveniles. To date, the AKCRRAB program is the first and only US aquaculture program to successfully demonstrate that king crabs can be cultured on a large-scale in a hatchery setting (Daly et al., 2009; Swingle et al., in review).

Biological and economic feasibility of hatchery culture is a significant challenge for red king crab stock enhancement, despite recent progress in culturing technology. Most crab and lobster stock enhancement programs require hundreds of thousands or even millions of individuals for release (Aiken and Waddy, 1995; Bannister and Addison, 1998; Secor et al., 2002; Comeau, 2006; Stevens, 2006a, 2006b; Zohar et al., 2008). Red king crab stock enhancement in Alaska will likely also require annual releases of millions to support a viable fishery (Stevens, 2006b). Hatchery production of juvenile red king crabs is limited by cannibalism and slow growth. Additionally, hatchery-cultured red

king crabs have no experience with seasonal cycles, predator avoidance techniques, or foraging for natural food items, which may induce behavioral and morphological deficiencies (Davis et al., 2004; Young et al., 2008). Cannibalism and slow growth can be mediated with artificial substrates, diet modification, and temperature (Stevens and Swiney, 2005; Daly et al., 2009; Stoner, 2009; Stoner et al., 2010a, 2010b); however, additional rearing technologies must be developed to improve hatchery efficiency for a large-scale red king crab stock enhancement program to be feasible.

Hatchery production does not ensure stock enhancement success. Past enhancement efforts often focused on hatchery production and numbers of individuals released, rather than optimizing post-release survival (Secor et al., 2002; Lorenzen, 2005; Stevens, 2006a). It is now understood that predation of released juveniles can be intense and limit the effectiveness of stock enhancement efforts (Bell et al., 2005, 2008; Hines et al., 2008), but experiments to evaluate predation susceptibility are often not conducted (Blankenship and Leber, 1995; Lorenzen et al., 2010). Factors such as behavioral competence, size-at-release, time-of-release, and release habitat impact post-release survival for other species (Bell et al., 2008), which must be evaluated for developing optimal release strategies for red king crab. Critical habitat requirements have been identified for red king crabs (Loher and Armstrong, 2000; Stoner, 2009; Pirtle and Stoner, 2010; Pirtle et al., in review); however, information on predators and other ecological requirements of juvenile red king crabs in nearshore habitats is scarce.

My research addresses some of the challenges described above, which is needed to develop a "responsible approach" and evaluate the feasibility of king crab stock

enhancement in Alaska. Chapter 1 examines effects of dietary supplementation on survival, growth, and shell coloration and explores potential for morphological plasticity of hatchery-cultured juvenile red king crabs. Chapter 2 examines size grading as a means to mitigate cannibalism during juvenile hatchery rearing and increase hatchery production. Chapter 3 explores the potential for behavioral plasticity of juvenile red king crabs by exposing crabs to predators and examining improved cryptic behavior. Chapter 4 examines predation of hatchery-cultured juvenile red king crabs in the wild to identify predator species in nearshore habitats and assess relative predation pressure during the first juvenile instar stages.

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CHAPTER 1:

Dietary Astaxanthin Supplementation For Hatchery-Cultured Red King Crab, *Paralithodes camtschaticus*, Juveniles[1]

ABSTRACT

We conducted large-scale production trials in Seward, Alaska, USA to investigate effects of dietary astaxanthin supplementation on survival, growth, and shell coloration of recently settled juvenile (C1-C4) red king crabs (*Paralithodes camtschaticus*). We supplemented a control diet of commercial crustacean feeds with astaxanthin, and fed these diets to juvenile king crabs at densities of 2000 and 4000 crabs m^{-2} surface area for 56 days. We assessed survival and growth by counting crabs and individually measuring carapace width and weighing crabs at the start and end of the experiment and quantified crab color (hue, saturation, brightness) in digital photographs. Diets containing astaxanthin had higher survival, suggesting that astaxanthin may provide nutritional or immune system benefits. Crabs had lower hue, higher saturation, and lower brightness values when fed diets containing astaxanthin suggesting that red king crab coloration is plastic and responds to diet. Astaxanthin is likely an important dietary component for hatchery or laboratory reared red king crab juveniles and should be considered for aquaculture and other rearing of this and possibly other crustacean species.

[1] Daly, B., Swingle, S., Eckert, G. In press. Dietary astaxanthin supplementation for hatchery-cultured red king crab, (*Paralithodes camtschaticus*), juveniles. Aquaculture Nutrition.

INTRODUCTION

The growing global demand for seafood has increased pressure on fish stocks (Pauly *et al.* 1998). Aquaculture and stock enhancement have been suggested as possible approaches to meet that demand by enhancing or restoring depressed fisheries (Lorenzen *et al.* 2010). Progress in crustacean aquaculture has allowed for stock enhancement of crab and lobster species worldwide (Bannister & Addison 1998; Agnalt *et al.* 1999; Secor *et al.* 2002; Davis *et al.* 2005a; Zmora *et al.* 2005; Stevens 2006b; Bartley & Bell 2008; Cheng *et al.* 2008). Despite this progress, applications of hatchery technology to stock enhancement are often limited by challenges including economic feasibility of juvenile production and post-release survival (Blankenship & Leber 1995; Bell *et al.* 2005; Lorenzen 2008).

Red king crab (*Paralithodes camtschaticus*, Tilesius 1815) was one of the most important fisheries in Alaska, USA; however, many populations remain depressed despite fishery closures (Stevens *et al.* 2001; Woodby *et al.* 2005). Stock enhancement has been proposed as a possible population recovery tool for red king crabs because they are some of the most commercially valuable crustaceans in the world, and recruitment limitation has been proposed to explain their lack of recovery in the absence of fishing (Blau 1986). The Alaska King Crab Research and Rehabilitation and Biology (AKCRRAB) program was created in 2006 to assess the feasibility of stock enhancement for king crabs in Alaska and expanded on previous rearing technologies (Nakanishi & Naryu 1981; Nakanishi 1987, 1988; Epelbaum *et al.* 2006; Kovatcheva *et al.* 2006; Stevens 2006a, 2006b). Since its inception, the AKCRRAB program is the first and only US aquaculture

program to successfully demonstrate that king crabs can be cultured on a large-scale in a hatchery setting (Daly *et al.* 2009).

As king crab stock enhancement through hatchery production becomes a possibility, it is increasingly important to understand ecological competence of artificially-reared juvenile red king crab. Artificial rearing conditions (i.e., artificial substrate, flow conditions, diet, lighting) may impact behavior and morphology, or reduce brain development as seen in other crustacean species (Sandeman & Sandeman 2000; Davis *et al.* 2005b). The literature is rich with examples of behavioral and morphological differences between hatchery and wild fish (see Brown & Day 2002; Huntingford 2004 for a review) and crustaceans (Davis *et al.* 2005b; van der Meeren 2005; Young *et al.* 2008). However, some behavioral or morphological deficiencies may be mitigated through conditioning or improved rearing conditions (Davis *et al.* 2005b; van der Meeren 2005; Le Vay *et al.* 2007; Young *et al.* 2008).

Hatchery-cultured juvenile red king crabs exhibit color variation and are generally lighter in color than wild crabs, which are deep orange/red (B. Daly & G. Eckert, pers. obs.; Fig. 1.1). Color of crustaceans is determined through two pathways, including morphological mechanisms and a physiological change via chromatophores (Rao 1985; Tlusty 2005). Morphological color change includes adjustments of pigments within the exoskeleton and tends to occur over a longer period due to variations in the amount and distribution of pigment and structure of the cuticle (Rao 1985; Robison & Charlton 2005). Atlantic rock crab (*Cancer irroratus*) and European green crab (*Carcinus maenas*) juveniles exhibit ranges of colors that correspond with surrounding benthic habitat

(Palma & Steneck 2001; Todd *et al.* 2006); however, the mechanism for this color variability is largely unknown. American lobsters (*Homarus americanus*) exhibit short-term color response to environmental cues such as background color and ultraviolet light in laboratory experiments (Tlusty *et al.* 2009). Additionally, lobster shell pigmentation is controlled by carotenoids that occur naturally in their diet (Tlusty 2005; Tlusty & Hyland 2005).

Astaxanthin is ubiquitous in marine environments because it is synthesized by phytoplankton and zooplankton and bio-accumulates throughout the food web (Andersson *et al.* 2003; Harmon & Cysewski 2008). Astaxanthin is the predominant carotenoid in many crustaceans (Herring 1972, 1973) and is incorporated into the body tissue as it moves from the digestive system to the epidermis, which adds a red hue to the overall shell color (Chien & Shiau 2005; Tlusty 2005; Tlusty & Hyland 2005; Barclay *et al.* 2006). Wild red king crabs feed on a wide range of invertebrate, vertebrate, and macroalgae species (Feder *et al.* 1980; Jewett & Feder 1982), suggesting that astaxanthin is likely a natural dietary component of juvenile red king crab. Specific advantages of dietary astaxanthin for red king crab are unknown; however, nutritional benefits (i.e., improved survival, growth, and coloration) may exist as observed in fish (Torrissen 1990) and other crustacean (Bordner *et al.* 1986; Howell & Matthews 1991; Dall 1995; Merchie *et al.* 1998) species.

Commercial crustacean feeds are not specifically formulated for red king crabs and yield varying survival and growth rates (Daly *et al.* 2009). Hatchery diets may lack critical nutritional components essential to juvenile red king crabs that are otherwise

found in natural diets. An improved feeding regime and diet may enhance shell coloration and optimize survival through superior nutrition, improved immunity (Babin *et al.* 2010) or decreased cannibalism by reducing pressure to seek additional nutrients (Brodersen *et al.* 1989; Borisov *et al.* 2007). The objective of this study was to test the effects of dietary astaxanthin supplementation on survival, growth, and shell coloration of juvenile red king crab under typical indoor hatchery grow-out conditions.

MATERIALS AND METHODS

Broodstock and larval rearing

Twenty ovigerous females were captured with baited commercial pots in Bristol Bay, Alaska during November 2008. Crabs were transported to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska and placed in 2000 L tanks (2.6 m^2 bottom surface area) containing flow through ambient seawater ranging from 3.3 to 8.3°C (mean ± SE 4.7 ± 0.1°C) and fed ad libitum (~20 g chopped herring and squid per crab twice $week^{-1}$ (~0.4% body weight d^{-1}). Once hatching began, larvae from each female were mixed together and raised in 1200 L cylindrical tanks for 50 days until the first juvenile instar (C1) stage. Larvae were fed enriched San Francisco Bay strain *Artemia* nauplii daily. *Artemia* nauplii were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media in 100 L cylindrical tanks for 24 h.

Experimental design

We initiated the experiment with 15,000 juvenile (C1) red king crabs, which were reared over a 56-day period starting on 1 June 2009. First juvenile instars (C1) were collected from larval rearing tanks shortly after settlement, mixed randomly and placed in containers, hereafter called silos. Each silo is a flat bottomed 58 cm tall by 58 cm diameter cylindrical container (~65 L volume) with a 100 μm mesh screen on the bottom (~0.25 m^2 surface area) (Daly *et al.* 2009). Ten silos were placed in each of two larger 3,200 L rectangular tanks. All silos were flow-through with water entering from the top with a flow rate of approximately 1.5 L min^{-1}(exchange rate h^{-1}=1.4). Incoming seawater was sourced from a deep-water (~75 m) intake at ambient temperature and was filtered to 5 μm, UV sterilized, and carbon filtered. Water temperature ranged from 8.2°C to 12.7°C (mean ± SE 9.6 ± 0.1°C). All silos contained equal amounts (approximately 100 g) of commercial fishing gillnet with a mesh size of 7.6 cm. The gillnet twine consisted of nine woven nylon monofilaments for a total diameter of approximately 1.0 mm and surface area of 88 cm^2 g^{-1}. The gillnet provided complex structure with interstitial spaces, and thus reduced crab contact with each other. Salinity was stable at 31-32‰. Two factors (diet and density) were varied resulting in 4 treatments that were each replicated five times. Stocking densities tested were 2000 and 4000 crabs m^{-2} surface area; the diet treatments are described below.

We fed crabs a control diet and one with astaxanthin supplementation. The control diet consisted of two ground (~50-400 μm particle size) cuttlebones (approximately 12 g each), the calcareous internal shell of a cuttlefish, mixed with 25 g

Zeigler™ shrimp feed (Zeigler Bros, Inc., PA, USA), and 25 g Otohime B1 (Reed Mariculture, CA, USA), which were bound with two egg whites (approximately 35 g each, composition: 88% water, 10% proteins, 2% carbohydrates, minerals, lipids (Yamamoto *et al.* 1997)). Cuttlebones contain approximately 85% calcium carbonate, of which 40% (~34% overall) is pure calcium (Birchall & Thomas 1983), for a total 418 g calcium kg^{-1} feed (dry weight). The astaxanthin supplement diet contained the same combination of feeds and egg whites, as described above, but contained 2 g dry powdered NatuRose™ (from *Haematococcus pluvialis* microalgae, Cyanotech Corp., HI, USA), which contains 1.5% pure astaxanthin (15,000 μg g^{-1}), resulting in approximately 380 μg astaxanthin g^{-1} feed (dry weight). This dose is comparable to other studies using dietary astaxanthin supplementation with shrimp and lobsters (D'Abramo *et al.* 1983; Barclay *et al.* 2006; Paibulkichakul *et al.* 2008). The diet mixtures were bound by cooking and then ground, producing moist particles approximately 400-1000 μm. Proximate composition of the diets was calculated based on reported manufacturer values (Otohime, Zeigler™, NatuRose™) and values from the scientific literature for egg white (Yamamoto *et al.* 1997) and cuttlebone (Birchall & Thomas 1983) (Table 1.1). We administered the control and supplement diets twice weekly, and both treatments were fed a maintenance diet on other days of the week. Because commercial feeds are not specifically formulated for red king crabs and yield varying survival and growth rates (Daly *et al.* 2009), we used a combination of commercial crustacean feeds as a maintenance diet. The maintenance diet consisted of alternating Cyclop-eeze®, Otohime B1 and B2, frozen enriched San Francisco Bay strain *Artemia* nauplii, and Zeigler™ shrimp feed. Cyclop-eeze® is a

frozen whole adult copepod (~800 μm length) high in carotenoids and omega-3 highly unsaturated fatty acids (HUFAs) and contains 50% protein and 35% lipid (Argent Chemical Laboratories, WA, USA). Otohime B1 (200-360 μm) and B2 (360-620 μm) is a high protein pellet feed developed for marine fish and contains 50% protein and 19% lipid (Reed Mariculture, CA, USA). Newly hatched *Artemia* nauplii (~400 μm length) contain approximately 50% protein and 19% lipid (Browne *et al.* 1991). *Artemia* nauplii were enriched with DC DHA Selco® enrichment media for 24 h to enhance their nutritional quality and then frozen. The frozen enriched *Artemia* nauplii (~750 μm length) were negatively buoyant and available for benthic crab consumption. Zeigler™ PL Redi-Reserve commercial shrimp feed (400-600 μm) is commonly used in crustacean aquaculture (Meade & Watts 1995) and contains 50% protein and 15% lipid (Zeigler Bros, Inc., PA, USA). All diets were administered at a rate of approximately 2% body weight (dry weight). Uneaten food was present and evenly dispersed in all treatments indicating food was not limiting and crabs were fed to satiation. Accumulated excess feed and waste were removed with a siphon weekly to maintain water quality.

Survival was assessed by counting all crabs within each replicate at the start and end of the experiment (day 1 and day 56). We assessed growth by individually weighing (blotted-dry wet weight) and measuring carapace width of ten randomly selected crabs from each replicate at the start and end of the experiment. Carapace width was measured with an ocular micrometer under 40x magnification. Carapace width was measured because the orientation of the small, motile crabs allowed more consistent measurements

than carapace length. Exuvia were examined to determine when molting to the next instar stage occurred.

We quantified color from digital photographs, using methodology similar to Davis *et al.* (2005b). Five randomly selected crabs from each replicate were photographed on a white background. A standardized photographic set-up was established to minimize differences in lighting. Crabs were placed in a small enclosed white container (~10 L) and photographs were taken under identical light conditions, camera, and laboratory location. Photographs were analyzed in Adobe Photoshop CS4 by standardizing the white background and identifying the hue (shade of color), saturation (amount of hue), and brightness (light vs. dark) of five random points on the carapace of each crab. Values were averaged to obtain one value of each color parameter per crab.

Statistical analysis

Factorial ANOVA and post-hoc comparisons (Tukey's HSD) were used to determine significance in survival, carapace width, wet weight, hue, saturation, and brightness values among diet and stocking density treatments (SigmaStat v.4, Aspire Software International, Ashburn, VA, USA). Survival data were arcsine square root transformed, while carapace width wet weight, and hue data were log transformed to meet assumptions of normality. Significance was determined using $\alpha = 0.05$.

RESULTS

Survival

Average (± SE) survival to day 56 across all treatments was 20.0 ± 1.5%. Survival varied significantly with astaxanthin supplementation but not with stocking density, and there were no significant interactions (Table 1.2, Fig. 1.2A). Survival was higher when astaxanthin was included in the diet (25.1 ± 1.7%) compared to without (15.0 ± 0.6%) (Table 1.2, Fig. 1.2A).

Growth

At the start of the experiment, C1 crabs were 1.73 ± 0.01 mm in carapace width and weighed 5.10 ± 0.12 mg. On day 56 crabs were a range of later juvenile instar stages (likely C2-C4s), with a carapace width of 2.71 ± 0.02 mm and an average wet weight of 21.10 ± 0.56 mg. The effect of astaxanthin on size of crabs measured by carapace width varied between density treatments (significant diet*density interaction, Table 1.2, Fig. 1.2B). Crabs supplemented with astaxanthin had larger carapace width at 2000 m^{-2} density (Tukey's HSD, $p<0.001$) but not at 4000 m^{-2} density (Tukey's HSD, $p=0.468$). Overall, crabs had larger carapace width when fed the astaxanthin diet (2.79 ± 0.04 mm) compared to the control diet (2.63 ± 0.03 mm) (Table 1.2, Fig. 1.2B, Tukey's HSD, $p<0.001$). Crabs were larger, as measured by wet weight, when fed astaxanthin but this varied with density (significant diet*density interaction, Table 1.2, Fig. 1.2C). Crabs had lower wet weight when fed the control diet at 2000 m^{-2} density compared to control diet at 4000 m^{-2} density (Tukey's HSD, $p<0.001$) and crabs fed the astaxanthin diet at both

densities (Tukey's HSD, p=0.004). The main effect of diet was significant but not that of density. Crabs had higher wet weight when fed the astaxanthin diet (22.40 ± 0.89 mg) compared to the control diet (19.80 ± 0.94 mg) (Tukey's HSD, p=0.012).

Coloration

Color hue varied as a function of astaxanthin supplementation but not density and there was no diet*density interaction (Table 1.3, Fig. 1.3A). The astaxanthin diet yielded lower hue values (20.8 ± 0.4°) compared to the control diet (27.2 ± 0.5°) (Tukey's HSD, p<0.001). Color saturation varied as a function of astaxanthin supplementation but not density and there was no diet*density interaction (Table 1.3, Fig. 1.3B). The astaxanthin diet yielded higher saturation values (48.7 ± 0.7%) compared to the control diet (46.1 ± 0.9%) (Tukey's HSD, p=0.016). Color brightness varied as a function of astaxanthin supplementation and density with a significant diet*density interaction (Table 1.3, Fig. 1.3C). Crabs fed the control diet at 2000 m^{-2} density (79.3 ± 1.2%) had lower brightness than at 4000 m^{-2} density (84.5 ± 0.6%) (Tukey's HSD, p<0.001). Crabs fed the control diet at both densities had higher brightness than crabs fed the astaxanthin diet at both densities (p<0.001 for all Tukey's HSD pairwise comparisons).

DISCUSSION

The present study demonstrates that dietary astaxanthin supplementation improves survival, growth, and color of hatchery-cultured juvenile red king crabs. Benefits of dietary astaxanthin are reported for other crustaceans, including Kuruma

prawns (*Marsupenaeus japonicus*), tiger prawns (*Penaeus monodon*), Pacific white shrimp (*Litopenaeus vannamei*), and American lobsters, which may be due to an enhancement of immunity, or salinity, thermal, and pathological stress resistance (Bordner *et al.* 1986; Darachai *et al.* 1998; Chien *et al.* 2003; Chein & Shiau 2005; Niu *et al.* 2009; Pei *et al.* 2009; Babin *et al.* 2010). Additionally, astaxanthin improves maturation rates and spawning success of tiger prawns (Paibulkichakul *et al.* 2008) and improves growth of several other prawn and lobster species (Bordner *et al.* 1986; Thongrod *et al.* 1995; Petit *et al.* 1997). In fishes, astaxanthin is suspected to improve provitamin A activity, act as an antioxidant, improve embryonic and larval development, protect from photodynamic damage, enhance growth and maturation, and facilitate oxygen reserves under anoxic conditions (Torrissen 1990). Suboptimal diets induce increased cannibalism of captive red king crabs, presumably to supplement nutritional deficiencies (Brodersen *et al.* 1989). Our result of increased growth (higher weight) at higher densities in the control diet but not in the astaxanthin diet may suggest that astaxanthin supplementation reduces cannibalism. Cyclop-eeze®, which was used in all treatments in our study, contains astaxanthin levels as high as 3,000 $\mu g\ g^{-1}$, and improves survival and astaxanthin content of freshwater prawns (*Macrobrachium rosenbergii*) (Nair *et al.* 2007). However, the level used in the astaxanthin supplemented diet is more concentrated (15,000 $\mu g\ g^{-1}$), suggesting that increased concentrations provide nutritional benefits. As such, high levels of astaxanthin should be included in rearing protocols to improve hatchery production and culturing efficiency of juvenile red king crabs and possibly other crustaceans.

Astaxanthin supplementation improves juvenile red king crab shell pigmentation. Because red king crabs cannot synthesize astaxanthin, it must be acquired through diet. Shrimp and lobster aquaculture use astaxanthin to manipulate pigmentation (Yamada *et al.* 1990; Liao & Chien 1994; Tlusty & Hyland 2005; Barclay *et al.* 2006). American lobsters require a specific amount of astaxanthin (approximately 100 μg pigment g^{-1} diet) to maintain their "wild" color (D'Abramo *et al.* 1983; Tlusty & Hyland 2005), while insufficient dietary astaxanthin levels result in a blue coloration (D'Agostino 1980; Lim *et al.* 1997). Tropical spiny lobsters (*Panulirus ornatus*) are also influenced by astaxanthin and become paler with decreasing dietary levels (Barclay *et al.* 2006). Administered astaxanthin levels were higher in the present study (380 μg pigment g^{-1} diet) than those reported by D'Abramo *et al.* (1983) and Barclay *et al.* (2006), thus it is not surprising that astaxanthin supplements produced crabs with darker coloration (lower hue and brightness values), as red king crabs likely have similar mechanisms for pigmentation as other crustaceans including lobsters. Without astaxanthin supplements, hatchery-cultured crabs may be obtaining inadequate amounts of carotenoids compared to wild crabs resulting in lighter color.

For stock enhancement programs, improving shell pigmentation of hatchery-cultured red king crabs may increase post-release survival. Reducing predator detection is critically important for recently settled red king crab survival. Generally, structural complexity facilitates physical crypsis by reducing predator encounter rates (Lima & Dill 1990), while camouflage facilitates visual crypsis (Palma & Steneck 2001). The darker shell color may increase crypsis by more closely resembling substrates that juvenile red

king crab are typically found such as rock, cobbles, and bivalve shells (Loher & Armstrong 2000), while the contrast of relatively lighter shell color on dark substrates may increase vulnerability to visual predators.

Mechanisms for color change in red king crab are unknown; however, the ability to change shell color through diet suggests that shell color is plastic and not entirely regulated by a genetic predisposition. Blue crabs (*Callinectes sapidus*) can change shell color in response to substrate color within one day (Davis *et al.* 2005b). Generally, short-term color change in response to light or background color variability occurs in crustaceans with chromatophores and translucent shells, which can mobilize pigments to change color (Ghidalia 1985). However, crustaceans such as American lobsters that have a thick calcified cuticle show color plasticity in response to background color (Tlusty *et al.* 2009). Whether red king crabs may exhibit color plasticity in response to varying background color is subject for future study.

Our previous juvenile red king crab hatchery experiments (Daly *et al.* 2009) yielded higher overall survival (50-60% compared to 15-30%) which can be attributed to a shorter rearing duration (42 days compared to 56 days) and colder rearing temperatures (7-10°C compared to 8-13°C). The daily mortality rates of the 2000 crabs m^{-2} density treatment was 1.3% mortality d^{-1} in the previous study and 1.8% mortality d^{-1} in this study, which is likely attributed to exacerbated cannibalism from the warmer rearing temperature (Stoner *et al.* 2010). The longer rearing period and warmer rearing temperature in this study allowed for additional growth. Crabs in the previous study were 2.05 mm carapace width after 42 days, whereas crabs in the current study were 2.71 mm

carapace width after 56 days, representing growth rates of 0.007 mm carapace width d^{-1} compared to 0.017 mm carapace width d^{-1}. These results suggest that rearing conditions were good in both studies. Crabs reared at densities of 500, 1000, and 2000 crabs m^{-2} had increased survival at lower densities (Daly *et al.* 2009), while the present study reared crabs at 2000 and 4000 crabs m^{-2} and found similar survival at both densities. We expected differential survival rates between these two high density treatments, yet the low survival at both densities suggests that 2000 crabs m^{-2} may be an upper threshold beyond which densities are suboptimal.

Astaxanthin is likely an important dietary component for red king crab and should be administered through dietary supplementation in future hatchery production or laboratory rearing. Supplementing commercial feeds with astaxanthin increases survival and yields darker shell pigmentation more comparable to wild crabs. While statistically significant, it is unknown if this change in color is biologically meaningful. For example, color may be linked to survivorship in the wild. Future studies should make behavioral and morphological comparisons between hatchery-cultured and wild crabs and investigate susceptibility of hatchery-cultured crabs to predation in the natural environment. Any morphological deficiencies that may increase susceptibility to visual predators such as pale coloration may be alleviated prior to release with appropriate dietary supplements improving efficiency of stock enhancement programs.

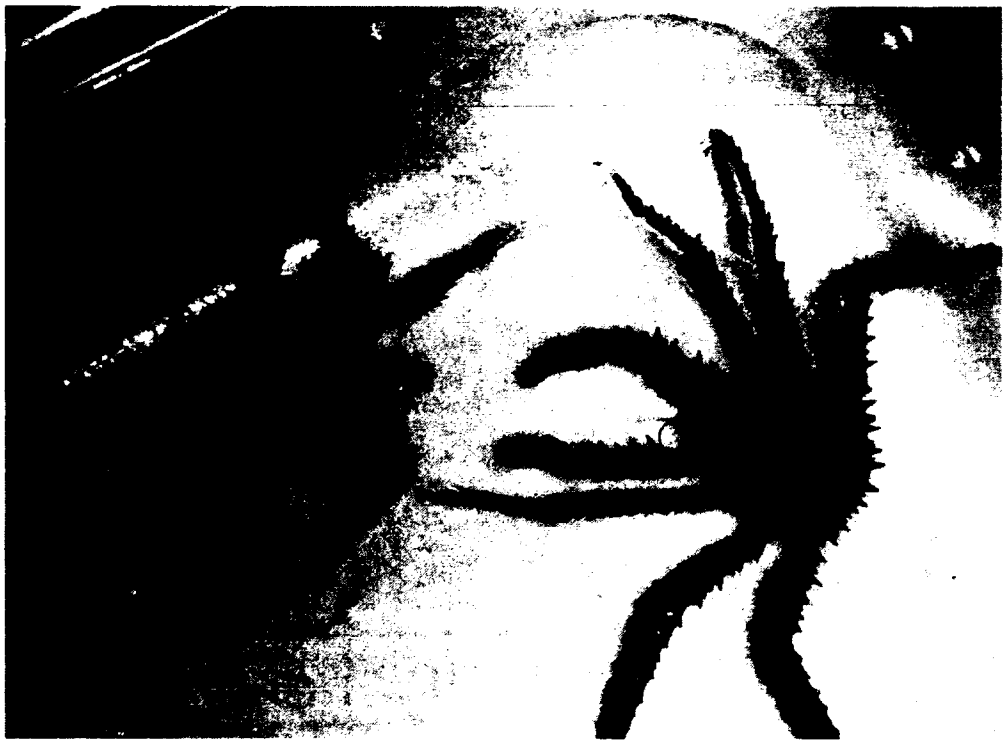

Figure 1.1. Color variation observed for two hatchery-cultured red king crabs. Color of wild red king crabs is comparable to the crab on the left. Photo by G.L. Eckert.

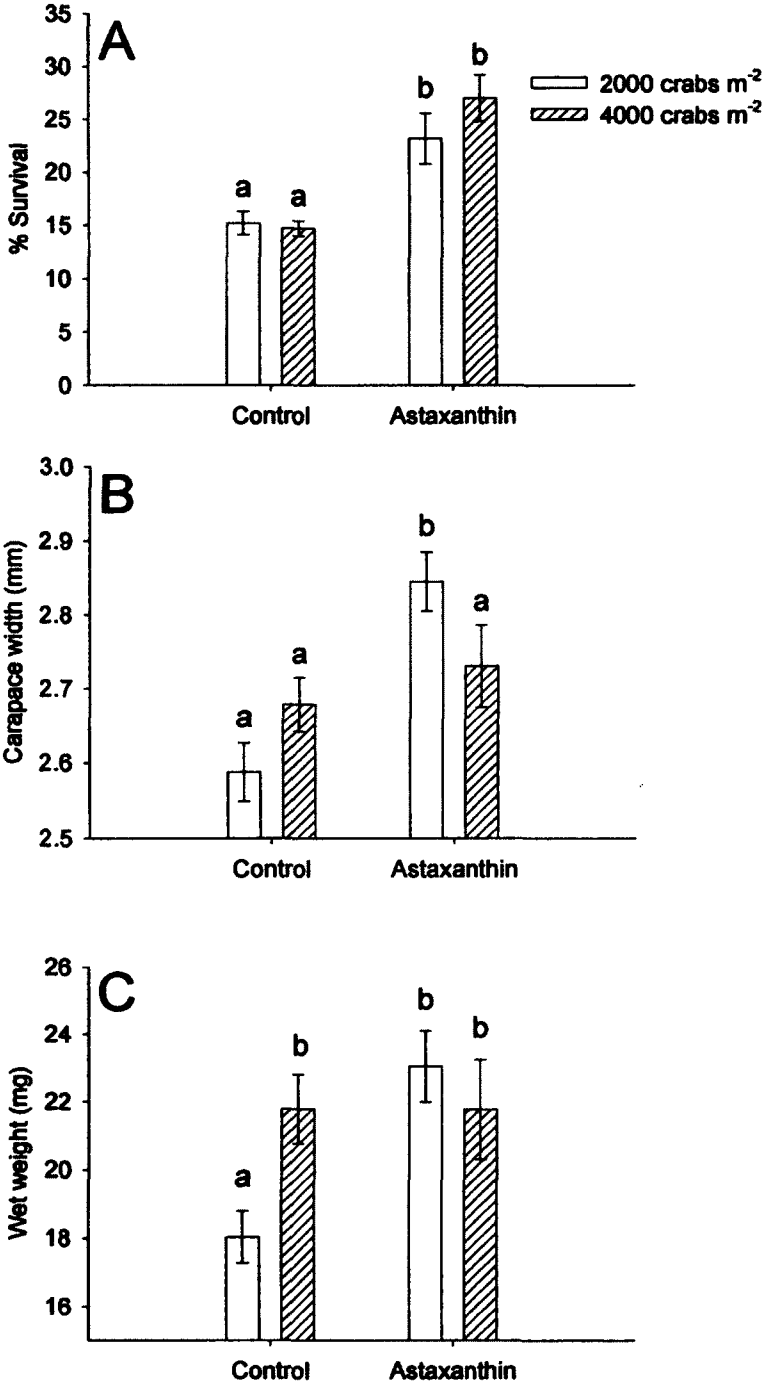

Figure 1.2. Mean ± SE for A) survival, B) carapace width, and C) wet weight for red king crab fed two diets (control, astaxanthin) and two stocking densities (2000 and 4000 crabs m^{-2}). Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

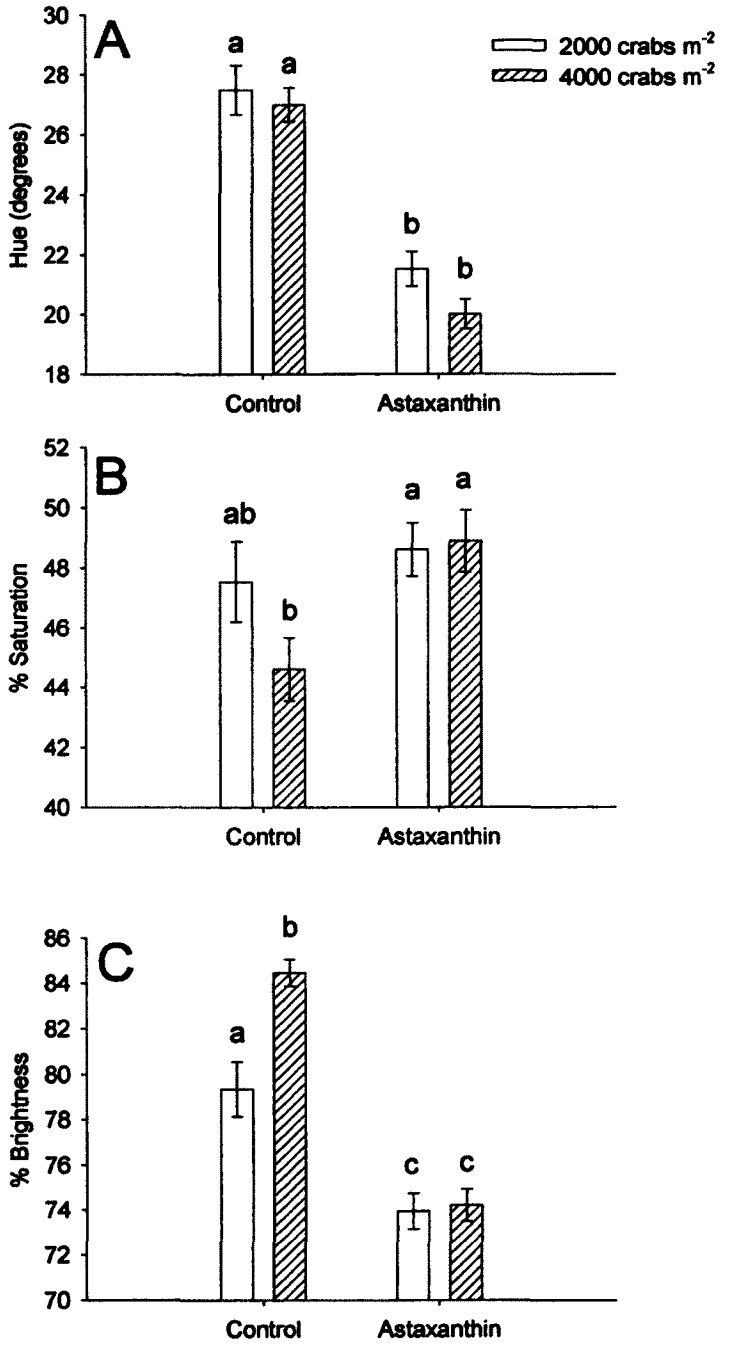

Figure 1.3. Mean ± SE for A) hue, B) saturation, and C) brightness for red king crab fed two diets (control, astaxanthin) and two stocking densities (2000 and 4000 crabs m^{-2}). Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

Table 1.1. Proximate composition in terms of percentage dry weight and ingredients for control and astaxanthin diets. The ingredients are identical between the two diets with the exception of the addition of NatuRose™ to the astaxanthin diet, which results in small variation in percentage dry weight for the other ingredients.

	Control	Astaxanthin
Proximte composition (percentage dry weight)		
Protein	41.8%	41.4%
Lipid	8.4%	8.6%
Ash	8.1%	8.3%
Fiber	1.9%	1.9%
Ca	10.5%	10.3%
Astaxanthin	0.000%	0.038%
Ingredients		
NatuRose™		2 g
cuttlebone	24 g	24 g
Otohime B1	25 g	25 g
Zeigler™	25 g	25 g
egg white (wet weight)	70 g	70 g

Table 1.2. Factorial ANOVA for survival, carapace width, and wet weight of red king crab (*P. camtschaticus*) juveniles. Bold indicates statistical significance ($\alpha \leq 0.05$).

	Effect	SS	*df*	MS	*F*	*p*
Survival	Diet	0.0797	1	0.0797	35.50	**<0.001**
	Density	0.0018	1	0.0018	0.81	0.382
	Diet x density	0.0031	1	0.0031	1.39	0.256
	Residual	0.0359	16	0.0023		
Carapace width	Diet	0.0283	1	0.0283	13.42	**<0.001**
	Density	0.0002	1	0.0002	0.10	0.755
	Diet x density	0.0147	1	0.0147	6.95	**0.009**
	Residual	0.3990	189	0.0021		
Wet weight	Diet	0.1220	1	0.1220	6.24	**0.013**
	Density	0.0218	1	0.0218	1.11	0.294
	Diet x density	0.1750	1	0.1750	8.94	**0.003**
	Residual	3.6880	188	0.0196		

Table 1.3. Factorial ANOVA for hue, saturation, and brightness of red king crab (*P. camtschaticus*) juveniles. Bold indicates statistical significance ($\alpha \leq 0.05$).

	Effect	SS	*df*	MS	*F*	*p*
Hue	Diet	0.348	1	0.348	107.23	**<0.001**
	Density	0.008	1	0.008	2.53	0.115
	Diet x density	0.004	1	0.004	1.26	0.264
	Residual	0.312	96	0.003		
Saturation	Diet	180.096	1	180.096	6.026	**0.016**
	Density	44.890	1	44.890	1.502	0.223
	Diet x density	64.964	1	64.964	2.174	0.144
	Residual	2869.130	96	29.887		
Brightness	Diet	1536.640	1	1536.640	83.71	**<0.001**
	Density	181.710	1	181.710	9.899	**0.002**
	Diet x density	146.894	1	146.894	8.002	**0.006**
	Residual	1762.250	96	18.357		

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CHAPTER 2:

Increasing Hatchery Production Of Juvenile Red King Crabs (*Paralithodes camtschaticus*) Through Size Grading[1]

ABSTRACT

Cannibalism is problematic in hatchery production of many crustaceans and can be exacerbated by differential growth, size variability, and asynchronous molting. We conducted two large-scale experiments in Seward, Alaska, USA to investigate effects of size grading on hatchery production (survival and growth) of juvenile red king crabs (*Paralithodes camtschaticus*). We reared larvae and subsequent juveniles until juveniles were eight weeks post-settlement. For each experiment, these eight-week old juvenile crabs were sorted into: "small" (<3.3 mm carapace width (CW)), "large" (>3.3 mm CW), and "ungraded" (approximately 2.0 to 4.5 mm CW) size classes. In the diet experiment, the three size classes were stocked at a density of 600 crabs m^{-2} and reared either on a control diet of commercial mariculture feeds or a control diet supplemented with astaxanthin and calcium for 53 days. In the density experiment, the three size classes were stocked at densities of 400, 900, and 1400 crabs m^{-2} and fed the control diet plus astaxanthin and calcium for 31 days. Survival in both experiments was strongly influenced by size grading. Generally, small crabs had higher survival than large and ungraded crabs. Diet was not a significant factor in growth or survival of crabs in the first

[1] Daly, B., Swingle, J., Eckert, G.L. In review. Increasing hatchery production of juvenile red king crabs (*Paralithodes camtschaticus*) through size grading. Prepared for publication in Aquaculture.

experiment. In the second experiment, large and ungraded crabs had higher growth and decreased survival with increasing density, likely from cannibalism. Small crabs had high survival and low growth at all stocking densities. These results suggest that larger juveniles must be held at reduced densities to maximize survival, while smaller individuals can be held at higher densities. Coupled with appropriate stocking densities, size grading should be used in laboratory and hatchery rearing protocols for red king crab and other likely cannibalistic crustaceans to maximize survival, improve hatchery efficiency, and increase the financial viability of large-scale stock enhancement or aquaculture programs.

INTRODUCTION

Aquaculture-based stock enhancement can be used to sustain or improve fisheries (Leber et al., 2004; Lorenzen et al., 2010); however, cannibalism is a bottleneck in the production of juvenile fish and crustaceans (Hecht and Appelbaum, 1988; Alston, 1991; Hecht and Pienaar, 1993; Aileen et al., 2000; Liao et al., 2001; Marshall et al., 2005; Zmora et al., 2005). Improvements in culturing technology are needed to help overcome cannibalism and improve the commercial viability of stock enhancement programs. Although not a direct artifact of artificial rearing, cannibalism is likely exacerbated by conditions associated with hatchery culture such as artificial diets, high stocking densities, absence of natural substrates, and elevated temperatures.

Cannibalism is widespread in the animal kingdom and occurs among both vertebrates and invertebrates (Fox, 1975; Polis, 1981). Stomach content analysis and field

surveys have documented cannibalism in various decapod crustacean species in the wild (Kurihara and Okamoto, 1987; Hines et al., 1990; Mansour and Lipcius, 1991; Fernández et al., 1993; Lovrich and Sainte-Marie, 1997; Marshall et al., 2005), showing that it is a natural behavior. Cannibalism may be intense during periods of strong recruitment and may control population dynamics of some crustacean species (Botsford and Wickham, 1978; Fernández et al., 1993; Lovrich and Sainte-Marie, 1997; Heck et al., 2001). For example, settling post-larvae and recently settled juveniles can be consumed by older conspecifics (e.g. *Hemigrapsus penicillatus* (Kurihara and Okamoto, 1987), *Cancer magister* (Fernández et al., 1993), *Callinectes sapidus* (Moksnes et al., 1997), *Chionoecetes opilio* (Lovrich and Sainte-Marie, 1997)) and conspecific mortality has been associated with Tanner crab (*Chionoecetes bairdi*) matting aggregations (Stevens et al., 1994). Generally, cannibals attack smaller individuals, presumably because they are more vulnerable and have weaker cuticles, while larger crabs have stronger chelae (Polis, 1981; Dutil et al., 1997, 2000; Luppi et al., 2001; Sainte-Marie and Lafrance, 2002). Recently molted individuals are more vulnerable to cannibalism; however, cannibalism can also occur during intermolt (Sainte-Marie et al., 1995; Dutil et al., 1997). Cannibalism associated with artificial rearing is more frequent in unfed compared to fed snow crabs (*Chionoecetes opilio*) (Dutil et al., 1997) and is coupled with increased growth rates for red king crabs (*Paralithodes camtschaticus*) (Brodersen et al., 1989; Borisov et al., 2007).

Size variation within a cohort is ubiquitous in fish and crustacean aquaculture and is likely influenced by a combination of genetic and environmental factors (e.g., social

interactions, rearing artifacts, food availability, temperature variability) (see Brett, 1979; Jobling and Baardvik, 1994; Wickens and Lee, 2002 for a review). High stocking densities associated with mass culture can result in strong resource competition among individuals. Size variation in prawns (*Macrobrachium rosenbergii*) is due to heterogeneous growth caused by aggressive interactions among morphotypes (Karplus et al., 1986). With many fish species, large individuals inhibit feeding efficiency of small fish causing a size hierarchy effect, where superior competitors obtain a disproportionate amount of food amplifying individual growth rate differences (see Brown, 1946; Magnuson, 1962; Noakes, 1978; Koebele, 1985 for a review). Variation in size among juveniles is not simply a result of cannibalism or agonistic interactions, as juvenile size can vary even when individuals are held in isolation, preventing social interactions. For example, molt duration and increment of individually cultured juvenile red king crabs varies within a cohort held within identical laboratory conditions (Westphal, 2011). Similarly, juvenile sunfish (*Elassoma evergladei*) vary in size and growth rate when reared in isolation (Rubenstein, 1981), suggesting genetic effects on growth.

Size grading (rearing small and large individuals separately) is commonly used in aquaculture of many species, including crabs (Marshall et al., 2005; Zmora et al., 2005), crayfish (Ahvenharju et al., 2005), freshwater prawns (Daniels and D'Abramo, 1994; Siddiqui et al., 1997; Tidwell et al., 2003), abalone (Mgaya and Mercer, 1995; Heath and Moss, 2009), eels (Karipoglou and Nathanailides, 2009), and fish (Carmichael, 1994; Barki et al., 2000; Wallat et al., 2005) to improve survival, growth, and feeding efficiency. Size grading reduces aggressive interactions by disrupting negative effects of

dominant, larger competitors that suppress the growth and survival of subdominant, smaller individuals (Karplus et al., 1986; Tidwell et al., 2003). Maintaining small individuals in the population may be important to conserve natural genetic and phenotypic variation (Frost et al., 2006), because some individuals may have a genetic predisposition for small sizes or slow growth rates (Gu et al., 1995; Frost et al., 2006). The genetic contribution of those individuals would be reduced if hatcheries select against slow growers. Regular size grading may increase survival or growth rates of smaller individuals from compensatory effects (Ricker, 1979; Jobling, 2010).

Most crab and lobster stock enhancement programs require hundreds of thousands or even millions of individuals for release (Aiken and Waddy, 1995; Bannister and Addison 1998; Secor et al., 2002; Comeau 2006; Stevens, 2006a, 2006b; Zohar et al., 2008). In Japan, annual production of juvenile swimming crabs (*Portunis trituberculatus*) is approximately 60 million with yearly releases ranging from 28 to 42 million (Secor et al., 2002). Stock enhancement has been proposed as a possible population recovery tool for depressed red king crab stocks in Alaska, USA and will likely also require annual releases of millions to support a viable fishery (Stevens, 2006b). Hatchery production of juvenile red king crabs is limited by cannibalism and slow growth, which can be mediated with artificial substrates, diet modification, and temperature (Stevens and Swiney, 2005; Daly et al., 2009, in press; Stoner, 2009; Stoner et al., 2010a, 2010b). Additional rearing technologies must be developed for a large-scale stock enhancement program to be economically feasible. This study aimed to determine the effects of size

grading by investigating survival and growth of size-graded juvenile red king crab reared using different diets and stocking densities.

MATERIALS AND METHODS

Broodstock and larval rearing

Twenty ovigerous females were captured with baited commercial pots in Bristol Bay, Alaska during November 2008 and 2009 for experiments the following spring and summer. Crabs were transported to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska, placed in 2000 L tanks (2.6 m^2 bottom surface area) containing flow-through ambient seawater, and each fed 20 g chopped herring and squid twice per week. Once hatching began (April 2009 and 2010), larvae from eight females were mixed and raised in 1200 L cylindrical tanks until the first juvenile instar (C1) stage. Zoeal larvae were daily fed San Francisco Bay strain *Artemia* nauplii, which were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media in 100 L cylindrical tanks for 24 h.

Nursery grow-out

We collected recently-settled, first stage (C1) juvenile crabs from larval rearing tanks, mixed them randomly and mass reared them in three 2000 L (2.6 m^2 bottom surface area) cylindrical nursery tanks for eight weeks. Nursery tanks contained artificial seaweed and commercial fishing gillnet (7.6 cm mesh size) to reduce agonistic interactions among conspecifics (Daly et al., 2009). Crabs were fed the control diet,

approximately 2% body dry weight, daily and excess feed and wastes were removed weekly. The control diet consisted of Cyclop-eeze® (Argent Chemical Laboratories, WA, USA), Otohime B1 and B2 (Reed Mariculture, CA, USA), frozen enriched *Artemia* nauplii, and Zeigler™ shrimp feed (Zeigler Bros, Inc., PA, USA), which where alternated daily. Cyclop-eeze® is a frozen copepod (~800 μm length) high in carotenoids and omega-3 highly unsaturated fatty acids (HUFAs). Otohime B is a high protein diet developed for marine fish and consists of 200-360 μm (B1) and 360-620 μm (B2) sinking pellets. Newly hatched San Francisco Bay strain *Artemia* nauplii (~450 μm length) have high levels of lipids and unsaturated fatty acids (Tizol-Correa et al., 2006). *Artemia* nauplii were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media for 24 h to enhance their nutritional quality and then frozen. The frozen enriched *Artemia* nauplii (~750 μm length) were negatively buoyant and available for benthic crab consumption. Zeigler™ PL Redi-Reserve commercial shrimp feed (400-600 μm particles) is commonly used in crustacean aquaculture due to its high levels of HUFAs (Meade and Watts, 1995).

Size grading

After the eight-week nursery grow-out period, crabs exhibited a size range of approximately 2.0 to 4.5 mm carapace width (CW). We collected crabs from mass rearing nursery tanks and sorted by size using a 3.3 mm mesh screen. We sorted equal numbers of crabs (~100) at a time to standardize any potential physical damage. Crabs that fell through the screen were assumed to be less than 3.3 mm CW, hereafter referred

to as "small". Crabs retained on top of the screen were assumed to be greater than 3.3 mm CW, hereafter referred to as "large." The ungraded treatment represented approximately 50% small and 50% large crabs.

Limb loss

To determine physical effects of the sorting process, we collected 100 pre-sorted crabs from the nursery tanks and examined them for missing limbs. The same crabs were then sorted by size using screens (described above) and reexamined for limb loss. The numbers of crabs with at least one missing limb and the total number of missing limbs per crab were recorded. The limb loss assessment was replicated three times.

Experiment 1: diet and size grading effects

We initiated the first experiment in summer 2009 after the eight-week nursery grow-out period. Two factors (three size-grading treatments and two diet treatments) resulted in six treatments that were each replicated six times. Crabs were sorted into small, large, and ungraded size classes as described above. Crabs were stocked at a relatively low density (600 crabs m^{-2}) in flat bottomed 58 cm tall by 58 cm diameter cylindrical containers with a 100 μm mesh screen on the bottom, a surface area of approximately 0.25 m^2, and volume of approximately 65 L, hereafter called silos. Ten silos were placed in each of four larger 3,200 L rectangular tanks. Size-grading treatments were randomly assigned among silos. All silos contained equal amounts (approximately 100 g) of commercial fishing gillnet (7.6 cm mesh size). The gillnet twine

consisted of nine woven nylon monofilaments for a total diameter of approximately 1.0 mm and surface area of 88 cm^2 g^{-1}. Gillnet improves survival by providing complex structure with interstitial spaces reducing crab contact with each other (Daly et al., 2009). All silos were supplied with flow-through ambient seawater entering from the top with a flow rate of approximately 1.5 L min^{-1}. Incoming seawater was sourced from a deep-water (~75 m) intake at ambient temperature and was filtered to 5 μm, UV sterilized, and carbon filtered. Temperatures ranged from approximately 8°C to 12°C.

Crabs were fed the control diet (described above) and the control diet supplemented with calcium and astaxanthin, which has been demonstrated to improve survival and growth of hatchery-cultured juvenile red king crabs (Daly et al., in press). Supplements consisted of two ground cuttlebones (~12 g each) and 2 g dry powdered NatuRose™ (1.5% pure astaxanthin) mixed with 25 g Zeigler™ shrimp feed and 25 g Otohime B1 and bound with 2 egg whites (~35 g each). Once bound with egg whites, supplements were ground producing particles approximately 400-1000 μm. Supplements were administered in lieu of the control diet twice weekly. Crabs were fed approximately 2% body dry weight daily and excess feed and waste were removed weekly.

The duration of the experiment was 53 days to allow crabs to molt at least once. Survival was assessed by counting all crabs within each replicate at the start and end of the experiment. Growth was assessed by weighing to the nearest 0.01 g (blotted wet weight) ten randomly selected crabs from each replicate at the end of the experiment.

Experiment 2: stocking density and size grading effects

We initiated the second experiment in summer 2010 after the eight-week nursery grow-out period. Crabs were size-graded as in Experiment 1. Crabs were placed in silos within rectangular tanks (described above) at densities of 400, 900, and 1400 crabs m^{-2}. Two factors (size-grading and density) were varied resulting in nine treatments that were each replicated four times. Crabs were fed the aforementioned mixed diet and reared at temperatures ranging from approximately 11°C to 14°C.

Experiment two was shorter in duration (31 days) due to a seawater pump malfunction; however, the warmer rearing temperature in 2010 likely allowed for crabs to molt at least once. Survival was assessed by counting all crabs within each replicate at the start and end of the experiment. Growth was assessed by weighing (blotted wet weight) all crabs within the same silo to give an aggregate total crab weight for each silo. The total crab weight was then divided by the total number of crabs within the silo to yield the average crab weight to the nearest 0.01 g.

Statistical Analysis

A paired t-test was used to determine significance in limb loss among pre- and post-sorted crabs. Two-way ANOVA and post-hoc comparisons (Tukey's HSD) were used to estimate significance in survival and weight among treatments. Weight data in experiment one were log transformed to meet assumptions of normality and equal

variance. All analyses were conducted using Sigma Stat v.4 (Aspire Software International, Ashburn, VA, USA). Significance for all tests was established with α=0.05.

RESULTS

Limb loss

We estimated only a small amount of limb loss as a result of the sorting process. After sorting, limb loss did not increase significantly in terms of percentage of crabs with at least one missing limb and the average number of missing limbs per crab (t=-2.392, *df*=2, p=0.139 for % missing limbs; t=-1.596, *df*=2, p=0.252 for missing limbs crab^{-1}). Prior to sorting, 22.7 ± 2.0% (average ± SE) of the crabs had at least one missing limb and 29.7 ± 4.0% had missing limbs after sorting (Fig. 2.1A). Crabs had 0.31 ± 0.02 missing limbs prior to sorting and 0.39 ± 0.06 missing limbs after sorting (Fig. 2.1B).

Experiment 1: diet and size grading effects

Average (± SE) survival to day 53 across all treatments was 54.4 ± 3.0%. Survival varied significantly among size-grading classes and marginally significantly between diets and there were no significant size-grading class*diet interactions (Table 2.1). Small crabs had higher survival (73.6 ± 3.5%) than large (50.9 ± 2.4%, Tukey's HSD, p<0.001) and ungraded crabs (38.9 ± 3.6%, Tukey's HSD, p<0.001) (Fig. 2.2A). Large crabs had higher survival than ungraded crabs (Tukey's HSD, p=0.024). Crabs fed calcium and astaxanthin supplements had marginally higher survival compared to crabs fed the control diet (Tukey's HSD, p=0.054).

Crab weight at the end of the experiment varied significantly by size-grading class but not by diet, and there were no significant size-grading class*diet interactions (Table 2.1). This was expected as crabs were pre-selected by size at the beginning the experiment. However, by day 53, large and ungraded crabs had a similar weight (Fig. 2.2B). Small crabs were lighter than large (Tukey's HSD, $p<0.001$) and ungraded (Tukey's HSD, $p<0.001$) crabs.

Experiment 2: stocking density and size grading effects

Survival and weight varied significantly by size-grading class and density with a significant size-grading class*density interaction (Table 2.2, Fig. 2.3A,B). The significant interaction reveals that survival and weight of the size grading classes were affected differentially by density. Small crabs were not affected by density (Fig. 2.3A,B, Tukey's HSD, $p>0.05$); whereas, increased densities resulted in lower survival and higher weights for large and ungraded crabs (Fig. 2.3A,B, Tukey's HSD, $p<0.05$).

DISCUSSION

Small crabs had higher survival than large and ungraded crabs and can be held at higher stocking densities without significant mortality. Differential survival rates likely resulted from size-related differences in behavior. Small (2-5 mm carapace length (CL)) red king crabs exhibit cryptic behavior and seek habitats with structural complexity (Stevens and Swiney, 2005; Stoner, 2009; Pirtle and Stoner, 2010), while relatively larger juveniles (e.g., 7.5-9.0 mm CL) become increasingly active in food searches, predator

response, and agonistic behavior with conspecifics (Pirtle and Stoner, 2010; Stoner et al., 2010b; B. Daly, pers. obs). Size-grading, by removing large, dominant crabs likely reduced competition for food, damage from aggressive interactions, and predation, while the cryptic and more docile nature of the small crabs likely facilitates high density holding due to reduced encounter rates. Increased activity of large and ungraded crabs may elevate agonistic interactions or cannibalism, especially at higher stocking densities. Large crabs had survival rates similar to ungraded individuals at moderate and high stocking densities, suggesting that large crabs do not necessarily prey on smaller conspecifics preferentially when held at elevated densities. Juvenile red king crabs may initiate cannibalism to eliminate competitive counterparts when resources (i.e., space, refuge availability) are limiting. As such, stocking density should be adjusted by crab size. For example, crabs less that 3.3 mm CW can be held at high densities with low mortality rates, while densities must be reduced for larger juveniles. In a hatchery setting, lower survival rates may achieve the goal of maximizing production per unit effort (Zmora et al., 2005; Ut et al., 2007), especially when considering rearing effort, utilization of hatchery space, and cost.

Recently molted crabs are at greatest risk of being cannibalized because of reduced mobility and lack of defensive armor. For blue-swimmer crabs (*Portunus pelgicus*), victims of cannibalism are recently molted and smaller than consumers, suggesting that transitional stages associated with ecdysis are especially vulnerable (Marshall et al., 2005). Early juvenile red king crabs (C1-C3) have relatively synchronous molt timing, while molting patterns become increasingly asynchronous with

time, causing a divergence in body size (Westphal, 2011; B. Daly and J. Swingle, pers. obs.). Smaller crabs, when compared to large crabs of the same age, may molt less frequently making them less vulnerable. Conversely, larger, faster growing crabs of the same age may molt more often leaving them more vulnerable to predation, which is exacerbated by asynchronous molting patterns and increased aggression. However, since crabs were held in populations, we cannot be certain if the observed size discrepancy is caused by variability in molting frequency or molt increment. For example, relatively small crabs may have similar molt frequencies as larger crabs of the same age, but have comparatively small molt increments. As such, size alone may be misleading when making assumptions regarding post-molt vulnerability. The degree of cannibalism in wild red king crab populations is unknown; however, we expect that high density hatchery-cultured crabs have much higher rates of cannibalism than what might be experienced in the wild.

Generally, crustacean metabolic demand is coupled with temperature resulting in increased feeding and growth (molt frequency, molt increment) with increasing temperature (Hartnoll, 1982; 2001). Recently-settled red king crab have a wide range of thermal tolerance (Stoner et al., 2010a); however, cannibalism is exacerbated with increasing temperature (Stoner et al., 2010b) likely from greater vulnerability associated with molting. Rearing temperatures in the present study (Exp 1: 8-12°C; Exp 2: 11-14°C) were likely warm relative to temperatures experienced by wild red king crab juveniles in northern areas (e.g., Norton Sound, Bristol Bay) but may represent temperatures in the Gulf of Alaska (e.g., Kodiak, Southeast Alaska). Because cannibalism is high during

molting, rearing temperature and experimental duration likely impacted our results. We estimate that crabs molted between one and three times during both experiments, but the longer experiment (Exp 1: 53 days) likely allowed a higher proportion of crabs to molt multiple times. Because the shorter experiment (Exp 2: 31 days) had a higher rearing temperature, experiments may have comparable growth. Culturing juvenile red king crabs at elevated temperatures does not decrease condition or nutritional status (Stoner et al., 2010a), suggesting benefits of temperature-mediated growth if cannibalism is minimized.

Large and ungraded crabs had lower survival but increased growth at higher stocking densities, which may result from competitive growth effects or enhanced feeding from social facilitation (Kurta, 1982). Additionally, greater cannibalism may have increased nutritional intake and growth of the survivors; however, uneaten food was present and evenly dispersed in all treatments indicating food was not limiting and equally available to all individuals. Density and diet dependent growth have been reported in previous studies using ungraded juvenile red king crabs (Daly et al., 2009; in press); however, the present study demonstrated growth did not increase in small-sized crabs at higher densities or with dietary supplements in any size treatment, suggesting that some crabs may have a genetic predisposition for slow growth regardless of diet and lack of competition from larger conspecifics. Because of likely genetic effects, we conclude that size grading is important for preserving a range of size classes, even when coupled with other rearing technologies such as optimal diet and increased structures.

Cannibalism in other species often occurs to meet metabolic demands when food is limiting (Elgar and Crespi, 1992); however, juvenile red king crabs exhibited

cannibalism when food was administered in excess. We report a marginally significant increase in survival with astaxanthin and calcium supplementation, which may have improved with greater replication. Previous studies with astaxanthin supplementation showed improved survival and shell coloration of ungraded red king crabs, suggesting astaxanthin provides some nutritional benefit (Daly et al., in press). Benefits of supplements may be amplified for ungraded crabs if subdominant individuals cannot obtain adequate nutrition with commercial feeds alone. Size grading may stabilize hierarchical structures and ease dominance pressure allowing crabs to feed satisfactorily in the absence of larger individuals. Astaxanthin is likely a natural dietary component of red king crab because it is synthesized by microalgae and bio-accumulates throughout marine food webs (Harmon and Cysewski, 2008). As such, astaxanthin supplementation should be included in hatchery diets especially in the absence of size grading.

Size grading increases total hatchery production of juvenile red king crabs and should be used in future rearing protocols in combination with other technologies such as artificial substrates, optimal diets, and appropriate stocking densities. By removing larger individuals, small crabs can be held at high densities without significant losses to cannibalism, while large individuals must be held at lower densities to achieve similar survival rates. As such, stocking density should be reduced as crab size increases. For red king crab stock enhancement, the optimal size for release is unknown and may require long-term hatchery grow-out. Improving nursery techniques will boost the productivity and financial viability of a large-scale stock enhancement program.

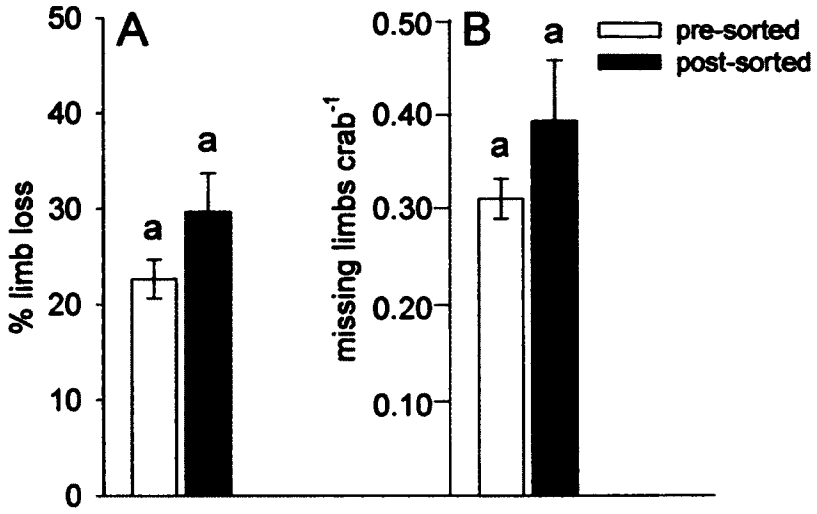

Figure 2.1. Average (± SE) (A) percent limb loss and (B) number of missing limbs per crab for crabs before and after they were sorted by size. Different letters indicate statistical significance (t test, $p \leq 0.05$).

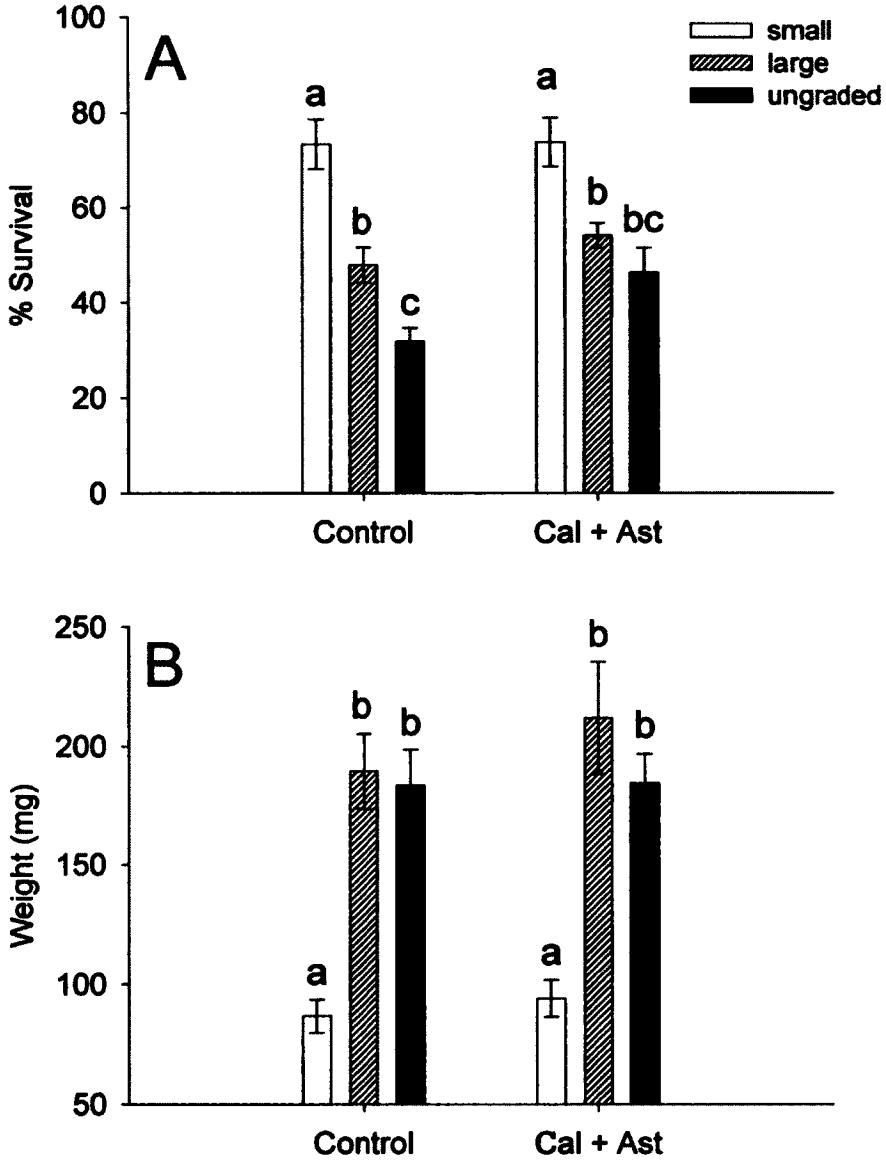

Figure 2.2. Experiment 1: Average (± SE) (A) percent survival and (B) weight of small (<3.3 mm CW), large (>3.3 mm CW) and ungraded juvenile red king crabs fed either the control diet or the control diet enriched with calcium and astaxanthin. Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

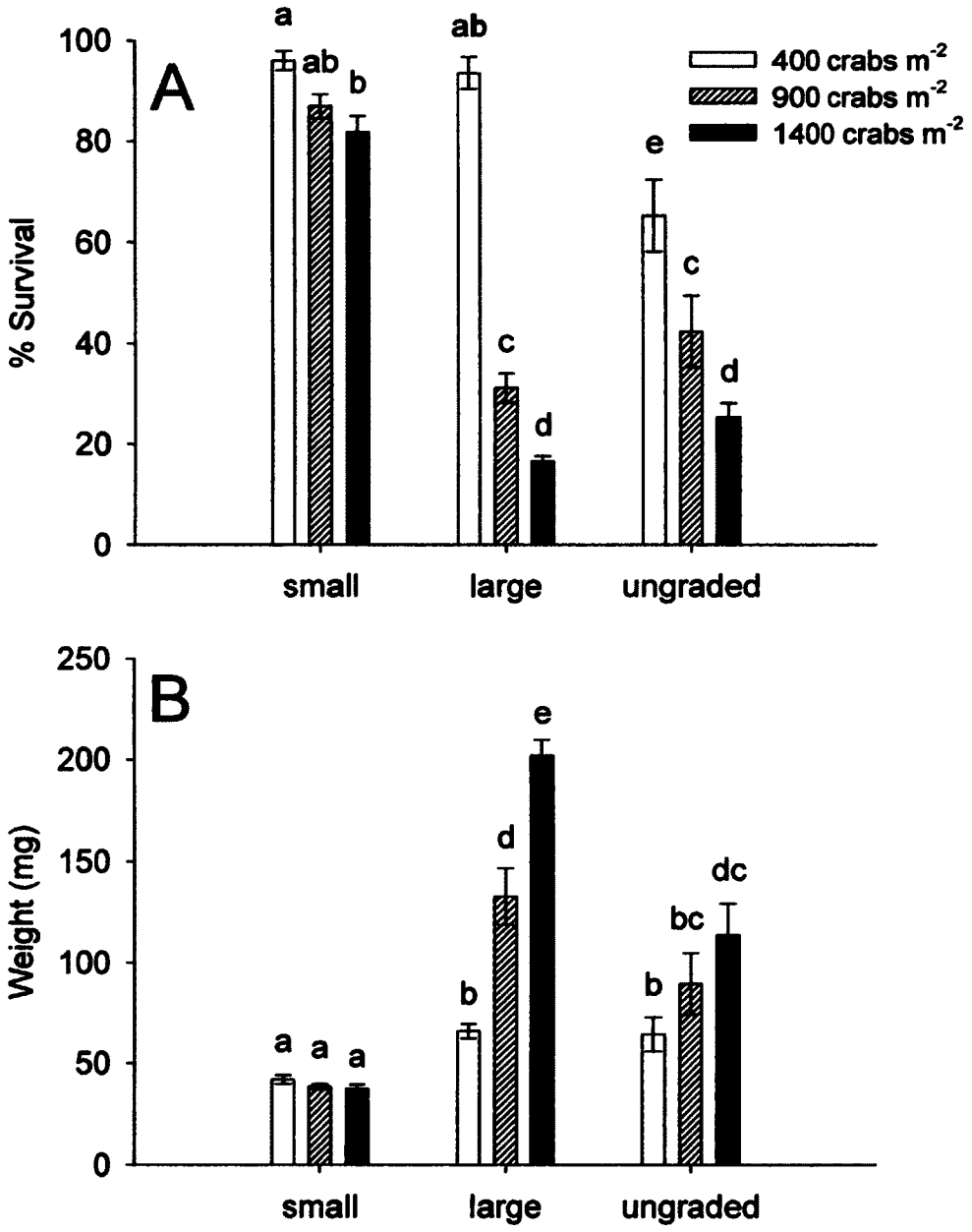

Figure 2.3. Experiment 2: Average (± SE) (A) percent survival and (B) weight of small (<3.3 mm CW), large (>3.3 mm CW), and ungraded juvenile red king crabs reared at 400, 900, and 1400 crabs m^{-2}. Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

Table 2.1. Experiment 1: Two-way ANOVAs for survival and weight of red king crab (*P. camtschaticus*) juveniles. Crabs were reared for 53 days with three size-grading classes (small, large, and ungraded) and two diets (control and control plus astaxanthin and calcium).

	Effect	SS	*df*	MS	F	*p*
Survival	Size	0.744	2	0.372	33.728	**<0.001**
	Diet	0.045	1	0.045	4.042	0.053
	Size x Diet	0.030	2	0.015	1.347	0.275
	Residual	0.331	30	0.011		
Weight	Size	4.510	2	2.255	51.028	**<0.001**
	Diet	0.042	1	0.042	0.943	0.333
	Size x Diet	0.006	2	0.003	0.067	0.935
	Residual	7.690	174			

Table 2.2. Experiment 2: Two-way ANOVAs for survival and weight of red king crab (*P. camtschaticus*) juveniles. Crabs were reared for 31 days among three size-grading classes (small, large, and ungraded) and three densities (400, 900, and 1400 crabs m^{-2}).

	Effect	SS	*df*	MS	F	*p*
Survival	Size	1.454	2	0.727	109.87	**<0.001**
	Density	1.221	2	0.611	92.29	**<0.001**
	Size x Density	0.480	4	0.120	18.14	**<0.001**
	Residual	0.179	27	0.007		
Weight	Size	53445.1	2	26722.5	71.81	**<0.001**
	Density	21777.4	2	10888.7	29.26	**<0.001**
	Size x Density	20164.0	4	5041.0	13.55	**<0.001**
	Residual	10048.2	27	372.2		

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CHAPTER 3:

Predator-Induced Behavioral Plasticity Of Juvenile Red King Crabs (*Paralithodes camtschaticus*)[1]

ABSTRACT

Crypsis is the primary predator-avoidance mechanism for recently-settled red king crabs (*Paralithodes camtschaticus*); however, it is unknown if this behavioral adaptation is innate or gained with experience. We conducted experiments with Pacific halibut (*Hippoglossus stenolepis*) and Pacific cod (*Gadus macrocephalus*) predators to determine if red king crab predator responses could be enhanced with experience. We exposed crabs to predators for 48 h either with limited exposure (chemical and visual cues only) or complete exposure (chemical, visual, and physical cues) and used video recordings to compare (1) crab crypsis, (2) crab survival, and (3) predator behavior (attack rates, capture success) among naïve and experienced crabs. Halibut exposure enhanced crab crypsis and survival, but cod exposure did not. Crabs with limited and complete halibut exposure had higher initial crypsis and both naïve and conditioned crabs increased crypsis by the end of the experiment. Complete exposure initiated a stronger response compared to limited exposure. Physical interactions with predators are likely important to initiate enhanced avoidance responses. Halibut and cod attack rates and capture success did not vary when crabs had prior experience, but halibut were generally

[1] Daly, B., Stoner, A.W., Eckert, G.L. In press. Predator-induced behavioral plasticity of juvenile red king crabs (*Paralithodes camtschaticus*). Journal of Experimental Marine Biology and Ecology.

more successful at capturing prey than cod. Our results show that juvenile red king crabs respond to some predators by increasing their cryptic behavior and that this response may be enhanced with experience. For stock enhancement programs, exposing red king crabs to predators in the hatchery prior to release may enhance predator avoidance and allow quicker adaptation to the natural environment.

INTRODUCTION

Predation is important for structuring communities in shallow marine environments. Many invertebrate species use predator-avoidance mechanisms (e.g., burial, camouflage, associating with structural complexity, immobility, temporal avoidance) to reduce detection or use predator-defense mechanisms (e.g., flight response, spination, chemical defenses, forming aggregations) to improve probability of survival once detected (Sih, 1987; Brodie et al., 1991; Barshaw et al., 2003). Most crustaceans use a combination of strategies to increase survival. For example, blue crabs (*Callinectes sapidus*) bury in soft sediment to avoid detection and deter predators with sharp lateral spines, while American lobsters (*Homarus americanus*) associate with specific substrates with interstitial spaces to avoid predators and have a flight response (tail flip), a thick calcified cuticle, and initiate aggressive claw displays (Lang et al., 1977; Hudon, 1987; Wahle and Steneck, 1991, 1992).

Crypsis is the primary predator-avoidance mechanism for recently-settled red king crabs (*Paralithodes camtschaticus*) (Stevens, 2003; Stevens and Swiney, 2005; Stoner, 2009; Pirtle and Stoner, 2010). Generally, early benthic phase (approximately age

0-2 years) juvenile red king crabs have a strong affinity for habitats with complex physical structures such as hydroids, polychaete tubes, bryozoans, shell hash, and cobble (Sundberg and Clausen, 1977; Dew, 1991; Loher and Armstrong, 2000) to avoid predation (Stevens, 2003; Stevens and Swiney, 2005; Stoner, 2009) or provide important foraging opportunities (Pirtle and Stoner, 2010). Crypsis diminishes with age (Pirtle and Stoner, 2010) and later stage juveniles (approximately 2 years old) begin to form aggregations or "pods" as a behavioral adaptation to minimize vulnerability to predators (Powell and Nickerson, 1965; Dew, 1990). Though we have a general understanding of habitat function for early red king crabs, we know relatively little about predator-prey interactions. For example, mechanisms for predator detection, predator-mediated habitat use, and whether or not behavioral adaptations are innate or gained with experience are unknown for red king crabs.

Stock enhancement has been used as a management tool for fish and invertebrate species worldwide and is being considered for red king crabs in Alaska, USA. Red king crab was one of the most important fisheries in Alaska; however, many populations remain depressed despite fishery closures (Stevens et al. 2001; Woodby et al. 2005). Though advancements in hatchery culturing technology have allowed for mass production of juvenile red king crabs, cannibalism is the primary obstacle limiting production (Stoner et al., 2010; Daly et al. in review). Hatchery-cultured animals have no experience with seasonal cycles, predator avoidance, or foraging for natural food items and rearing in isolation may exacerbate any abnormal behavioral development. Post-release survival will depend on an individual's ability to compete for resources, find

shelter, and avoid predation. Higher predation of hatchery-cultured compared to wild individuals occurs for fish (Kellison et al., 2000; Stunz and Minello, 2001) and invertebrate (Schiel and Welden, 1987; Stoner and Davis, 1994; Davis et al., 2004) species and is generally attributed to reduced ecological competence.

The literature is rich with examples of behavioral and morphological differences between hatchery and wild fish (see Ruzzante, 1994; Huntingford, 2004; Brown and Day, 2002 for a review) and crustaceans (Davis et al., 2005; van der Meeren, 2005; Young et al., 2008). For example, hatchery-cultured blue crabs are lighter in color and have shorter spines than wild crabs (Davis et al., 2005); while artificially reared European lobsters (*Homarus gammarus*) have abnormal claw development (Wickens, 1986; Tveite and Grimsen, 1995; van der Meeren, 2005). Reduced predator responses are noted with hatchery lobster (Castro and Cobb, 2005), crabs (Davis et al., 2004), fish (Olla and Davis, 1989; Kellison et al., 2000), abalone (Schiel and Welden, 1987), and conch (Delgado et al., 2002). Abnormal development acquired during hatchery culture may affect an individual's ability to avoid predators in the natural environment.

Behavioral or morphological deficiencies can be mitigated through conditioning or improved rearing conditions (Davis et al., 2005; van der Meeren, 2005; Le Vay et al., 2007). For example, European lobsters are more efficient at seeking cover with prior shelter experience (van der Meeren, 2001) and exposure to shell spat or coarse sand allows the development of natural claw proportions (Wickens, 1986; van der Meeren and Uksnøy, 2000). Conditioning with natural sediments also enhances color and burial behavior of blue crabs (Davis et al., 2004; Young et al., 2008), and predator exposure

improves burial behavior of queen conch (*Strombus gigas*) (Delgado et al., 2002) and increases survivorship of hatchery-cultured fish (Olla and Davis, 1989; Berejikian, 1995; Brown and Smith 1998; Kellison et al., 2000; Hossain et al., 2002). These results suggest that hatchery-reared animals have behavioral and phenotypic plasticity that can be initiated through exposure to specific environmental parameters. Conditioning red king crabs with predators may enhance adaptive behaviors such as crypsis and affinity for structural complexity or improve ability to detect and recognize threats.

We aimed to estimate conditioning potential of red king crabs by determining if predator responses could be enhanced with experience. We conducted laboratory predation trials and used video recordings to compare naïve crabs (no predator exposure) with crabs that had prior predator exposure (chemical and visual cues only or complete physical contact) in terms of (1) crypsis, (2) survival, and (3) predator feeding efficiency. We hypothesized that (1) crypsis and survival of red king crabs could be improved with predator experience and (2) predator feeding efficiency is reduced when crabs have predator experience.

MATERIALS AND METHODS

Experimental animals

We cultured juvenile red king crabs as part of the Alaska King Crab Research Rehabilitation and Biology (AKCRRAB) program using established rearing techniques (Daly et al., 2009). Twenty ovigerous females were captured with pots in Bristol Bay, Alaska USA during November 2009. Crabs were transported to the Alutiiq Pride

Shellfish Hatchery in Seward, Alaska and placed in 2000 L tanks (2.6 m^2 bottom surface area) containing flow through ambient seawater and fed 20 g chopped herring and squid per crab twice per week. Once hatching began (spring 2010), larvae from each female were mixed and raised in 1200 L cylindrical tanks until the first juvenile instar stage. Larvae were fed enriched San Francisco Bay strain *Artemia* nauplii daily. *Artemia* nauplii were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media in 100 L cylindrical tanks for 24 h. Juvenile crabs were held *en masse* in large-scale rearing tanks at the Alutiiq Pride Shellfish Hatchery until shipment to the Hatfield Marine Science Center (Newport, Oregon) in September 2010. In Newport, crabs were held *en masse* in 90 L tanks with flow-through seawater (8°C) and fed Cyclop-eeze® (Argent Chemical Laboratories, WA, USA) and Otohime B1 and B2 (Reed Mariculture, CA, USA) daily. Initial densities were ~500 crabs m^{-2}, with clumps of PVC ribbon (4 mm wide) (Bio-Fill filter medium) added for vertical structure.

Pacific halibut (*Hippoglossus stenolepis*) and Pacific cod (*Gadus macrocephalus*) are effective predators of juvenile red king crabs in the laboratory (Stoner, 2009; Pirtle and Stoner, 2010; Pirtle, 2010) and were used as predators in two separate sets of experiments, which were conducted in compliance with Institutional Animal Care and Use Committee (IACUC) protocols (approved October 19, 2010, project: 186691-3). Age-1 Pacific halibut (185 mm total length) and age-1 Pacific cod (205 mm total length) were collected as age-0 fish from Chiniak Bay, Kodiak Island, Alaska (collection permit: CF-09-081) and grown to this size at the Hatfield Marine Science Center in Newport, Oregon. Fish were fed primarily gel food prepared from squid and herring, and

maintained in flow-through seawater in large circular tanks for ~12 months before being used in crab predation experiments.

Crab conditioning

Crabs were either "conditioned with limited exposure," "conditioned with complete exposure," or "naïve." We conditioned crabs by holding batches of approximately 40 individuals in three separate circular (103 cm diameter), flat-bottomed tanks containing three fish predators (either Pacific halibut or Pacific cod) per tank for 48 h prior to each experimental trial. Crabs conditioned with limited exposure were enclosed in a clear, acrylic column (28 cm diameter) with abundant (~0.5 per cm^2) 3 mm holes in the sides, allowing both visual and chemical cues but no physical contact with fish predators. The column included substrate (natural sand and hydroid mimics) for protection. Crabs conditioned with complete exposure were held in the same tanks as described above (with substrate) but without the acrylic column allowing physical interactions with fish predators. Naïve crabs were held in tanks similar to those above (with substrate) except the tanks lacked fish predators. Crabs were fed Cyclop-eeze® (Argent Chemical Laboratories, WA, USA) and Otohime C1 (Reed Mariculture, CA, USA) to satiation every other day.

Experimental apparatus

Immediately following the 48 h conditioning period, nine predation trials were conducted in three identical circular, flat-bottomed tanks and supplied with continuous

flows (150 ml s^{-1}) of sand-filtered seawater at 8.0°C (± 0.5°C). The bottom of each tank was covered with 1 cm of coarse-grained quartz sand (1.5 mm mean diameter). The tanks were in a light-controlled room with a daily light cycle of 12 h light and 12 h dark (0700 to 1900 h). The daytime light level was 3 μmole photons m^{-2} s^{-1} provided by a bank of fluorescent lamps around the upper periphery of the room that was controlled by a rheostat. This allowed light levels to be lowered to darkness (<1 x 10^{-8} μmole photons m^{-2} s^{-1}) then raised slowly during experimental trials to prevent startling the predators. Each tank was equipped with an overhead video camera monitored from an adjacent room to assure that the tanks were undisturbed during trials. A clump of hydroid mimics was placed in the center of each sand-covered tank bottom to provide structural complexity and refuge for the crabs. The hydroid mimic clump was made of 50 strands (20 cm length) of brown polyester and was allowed to foul in flowing seawater for at least 2 weeks prior to experimentation. Fouled hydroid mimics are preferred substrates for age-0 juvenile red king crabs (Pirtle and Stoner, 2010), and their consistent dimensions allow for standardized volume of substrate. Trials using halibut as predators were conducted in tanks 103 cm diameter x 25 cm depth, while trials using cod as were conducted in larger tanks (140 cm diameter x 75 cm depth).

Experimental protocol

Nine fish pairs (either Pacific halibut or Pacific cod) were transferred to the experimental tanks (separate from conditioning tanks) one week prior to the first trials to acclimate to the new surroundings. Hunger levels of fish were standardized by depriving

fish of food for 48 h prior to trials to insure that fish were uniformly motivated to feed. At the end of the week, fish pairs were presented with 10 age-0 red king crabs (5.0-7.5 mm carapace length) as prey to ensure that the fish were motivated to forage on red king crabs as prey in the experimental system. Pairs of fish were used because they perform more consistently as predators with social facilitation (Stoner and Ottmar, 2004). Fish were fed frozen krill (*Euphausia pacifica*) to satiation after trials.

On the morning (0800 h) of a predation trial, the lighting in the room was slowly reduced to total darkness. Twenty randomly selected juvenile red king crabs (5.0-7.5 mm carapace length) from each conditioning tank (naïve, conditioned with limited exposure, conditioned with complete exposure) were dispersed over the hydroid mimic island in each of three separate experimental tanks. Crabs were confined to the hydroid mimics with an acrylic column to ensure that the crabs settled into preferred microhabitats. The column was removed after 15 min and the crabs were able to access the entire tank for an additional 45 min (still in total darkness). At 0900 h, video recording was started and room lights were returned to standard illumination (approximately 3 μmol photons m^{-2} s^{-1}) over a period of about 60 s. The room was fully illuminated and the trial was recorded. Halibut trials lasted 60 min, while cod trials lasted 240 min because cod are less enthusiastic predators. Halibut and cod trials were replicated nine times. At the end of each trial, the sediment was thoroughly searched for crabs to determine survival rates. Crabs were not reused in subsequent trials. Each of nine fish pairs received each of the three treatments (naïve, conditioned with limited exposure, conditioned with complete

exposure) once in a randomized block design. Three additional trials were conducted without fish predators to control for cannibalism.

Statistical Analysis

Halibut experiment

The proportion of the twenty crabs alive at the end of each trail was used to calculate survival. Video recordings were reviewed for fish and crab behavior by dividing the entire 60-min trial into 5-min blocks of time. A crab crypsis index was calculated by counting the numbers of crabs visible on the sand and the top of the hydroid mimics at the start of each 5-min block. The proportion of live crabs not visible was then used as a crypsis index. We adjusted the crab crypsis index for predation by subtracting crabs eaten from the total number of crabs at the start of each 5-min block. It was assumed that crabs not visible were located within the hydroid mimics and were displaying a cryptic behavior, which was confirmed at the end of each trial. We quantified fish behavior using methodology similar to Stoner (2009). A fish activity index was calculated by summing the number of fish that actively moved during each 5-min block (possible range = 0-24), divided by the total possible ((60-min trial / 5-min blocks) x 2 fish = 24) for the percent of maximum. Three of the nine halibut pairs had an activity index of less than 0.25 and were excluded from analyses because they did not actively move around the tank and were assumed to be uninterested in foraging. Halibut typically had multiple strikes on individual crabs targeted for consumption and individual targets (crabs) were not always successfully consumed. Individual crabs targeted by fish and total number of strikes on

crabs were counted for each 5-min block. From this, total targets, targets per min, fish strikes per crab, crabs eaten per number of crabs targeted, and crabs eaten per fish strike were calculated for each trial. Differences in fish behavior (activity, attack rates, capture success) and crab behavior (survival, crypsis) among treatments (naïve, conditioned with limited exposure, and conditioned with complete exposure) were determined with randomized block ANOVA where conditioning treatment (n=3) was a fixed factor and fish pair (n=6) was a random blocking factor. Tukey's post-hoc comparisons were applied to test for differences among treatments. After visual inspection of the crab crypsis data, non-linear (quadratic) regression analysis was used to determine changes in crypsis over time using the model (crab crypsis index = A + B*time + C*time^2) because it appeared to describe the data better than linear regression.

Cod experiment

Video recordings were sub-sampled and reviewed for fish and crab behavior by dividing the first 15 min of each hour into 5-min blocks of time. From this, targets per min and strikes per individual crab targeted were calculated for each trial. Fish activity was quantified by summing the instances when an individual fish crossed from one quadrant of the circular tank (in the video analysis) to another for the first 5 min of each hour. The number of strikes and crabs targeted from sub-samples were extrapolated to the total trial time (240 min) to determine crabs eaten per target and crabs eaten per fish strike. Differences in fish behavior (activity, attack rates, capture success) and crab survival among naïve, conditioned with limited exposure, and conditioned with complete

exposure treatments were determined with randomized block ANOVA and Tukey's post-hoc comparisons, as with halibut trials. Cod trials were conducted in larger tanks, which prevented individual crabs from being visible in the video recordings and did not allow crab crypsis to be calculated.

Halibut-cod behavioral comparison

The halibut-cod behavioral comparison was conducted by pooling fish behavioral parameters (crabs targeted per min, strikes per target, crabs eaten per target, crabs eaten per strike) across conditioning treatments and using t-tests. Data were square root transformed to meet assumptions of normality and equal variance. All analyses were conducted using Sigma Stat v.4 (Aspire Software International, Ashburn, VA, USA) statistical software. Significance for all tests was determined with α=0.05.

RESULTS

Halibut experiment

Red king crab survival increased with conditioning (ANOVA, F=4.618, p=0.038) (Fig. 3.1A). Crabs conditioned with complete exposure had higher survival (average ± SE) (79.5 ± 8.5%) than naïve (61.2 ± 5.3%) crabs (Tukey's HSD, p=0.031) but not crabs conditioned with limited exposure (71.5 ± 6.4%) (Tukey's HSD, p=0.415). Crypsis index increased over time for all treatments following the non-linear equation of best fit: crab crypsis index = A + (B*time) + (C*time^2) (Fig. 3.2, Table 3.1). At t=0 min, naïve crabs had a lower crypsis index (40.5 ± 5.2) than crabs conditioned with limited exposure (60.8

± 5.0, Tukey's HSD, p=0.004) and crabs conditioned with complete exposure (76.5 ± 2.7, Tukey's HSD, p<0.001) (Fig. 3.3A). Crabs conditioned with limited exposure had a lower crypsis index than crabs conditioned with complete exposure (Tukey's HSD, p=0.020) (Fig. 3.3A). By t=60 min, crypsis of naïve crabs started to converge with that of conditioned crabs. At t=60 min, crypsis was not statistically different among naïve crabs (87.1 ± 5.5) and crabs conditioned with limited (95.3 ± 2.5) and complete (100.0 ± 0.0) exposure (ANOVA, F=3.420, p=0.074) (Fig. 3.3B). Control trials (no predators) had 100% survival indicating that cannibalism was not occurring.

Fish activity index, the number of crabs targeted, targets min^{-1}, crabs eaten $target^{-1}$, crabs eaten $strike^{-1}$, and strikes $target^{-1}$ were not impacted by crab conditioning level (Table 3.2). The number of crabs targeted was positively related with fish activity for naïve (regression, R^2=0.943, p<0.001), conditioned with limited exposure (regression, R^2=0.644, p=0.009) and conditioned with complete exposure (regression, R^2=0.897, p=0.002) treatments.

Cod experiment

Average crab survival was not statistically different among naïve crabs (67.7 ± 8.0%) and crabs conditioned with limited (80.1 ± 6.4%) and complete (79.3 ± 7.8%) exposure (ANOVA, F=1.904, p=0.181) (Fig. 3.1B). Fish activity (line crossings min^{-1}), the number of crabs targeted (extrapolated from sub-samples), targets min^{-1}, crabs eaten $strike^{-1}$, and strikes $target^{-1}$ were similar among conditioning treatments (Table 3.2); however, crabs eaten $target^{-1}$ was significant with fewer crabs eaten per target when crabs

were conditioned with limited exposure (0.10 ± 0.03) compared to naïve (0.19 ± 0.06) (Tukey's HSD, p=0.045) but not when conditioned with complete exposure (0.16 ± 0.05) (Tukey's HSD, p=0.165). Control trials (no predators) had 100% survival.

Halibut-cod behavioral comparison

Fish behavior (crabs targeted per min, strikes per target, crabs eaten per target, crabs eaten per strike) did not vary with respect to crab conditioning (Table 3.2), thus conditioning treatments were pooled for each of the above mentioned behavioral parameters. Crabs targeted min^{-1} and strikes $target^{-1}$ were similar between halibut and cod (targets min^{-1}: t test, t=-0.582, p=0.565; strikes $target^{-1}$: t test, t=-0.553, p=0.588) (Fig. 3.4A,B). Halibut consumed more crabs per target (0.70 ± 0.07) than cod (0.15 ± 0.03 crabs $target^{-1}$) (t test, t=6.144 p<0.001) and more crabs per strike (0.53 ± 0.07) than cod (0.14 ± 0.03 crabs $strike^{-1}$) (t test, t=5.012, p<0.001) (Fig. 4C,D).

DISCUSSION

Predator detection and avoidance

We initiated a behavioral response in juvenile red king crabs by exposing individuals to visual, chemical, and physical cues from fish predators. Crabs with prior halibut exposure had higher crypsis and survival than naïve crabs. Predator exposure likely increased affinity for physical structure as crabs were located deeper within the hydroid mass, while naïve crabs were mostly located near the periphery or on the open sand. Halibut are visual ambush predators, thus the hydroid mimic structure likely

reduced detection, while crabs near the periphery or on the open sand were more vulnerable. Other findings support our finding that red king crabs respond to predators through mediated habitat use. For example, Stevens and Swiney (2005) found that recently-settled red king crabs in experimental tanks moved to complex structures when larger conspecifics were added as predators. Additionally, early benthic phase red king crabs had a higher affinity for structure when fish predators (Pacific halibut) were present than when predators were absent (Stoner, 2009), and this response improved with crab age (Stoner et al., 2010). Further, tethered early benthic phase red king crabs maintain crypsis when attacked by predators in the field by remaining motionless in structural complexity (Pirtle, 2010).

An energetic trade-off may exist if predator avoidance causes reduced foraging (Abrahams and Dill, 1989; Wahle, 1992). Red king crab foraging activity may increase risk of predation, while crypsis may impede nutritional intake if foraging opportunities are limited either by reduced activity or by the choice of substrates with suboptimal nutritional quality. Because complex habitats provide foraging opportunities for juvenile red king crabs (Pirtle and Stoner, 2010), some habitat associations may allow crabs to remain cryptic without compromising nutritional intake.

Juvenile red king crabs likely detect predators via a combination of chemical, visual, and physical cues. Crabs exposed to visual and chemical cues alone had higher initial crypsis than naïve crabs but complete physical contact initiated an even stronger response. Fish predators could actively attack and consume crabs in conditioning tanks lacking physical barriers. Injured or damaged conspecifics likely created chemical signals

exacerbating cues from predators themselves; however, predation during the exposure period was minimal (~5-10% mortality). Alternatively, some crabs that lacked predator responses may have been consumed during the exposure period, thereby pre-selecting superior crabs for experimental trials. Because we detected a behavioral response with predator visual and chemical cues alone, we suggest that responses from complete exposure were not completely the result of a pre-selection bias, but rather that the exposure of crabs to fish predators increased their behavioral response to predators. Threatening stimuli are known to induce behavioral responses in other crustacean species. For example, American lobsters increase their habitat use (Wahle, 1992) and hermit crabs move away (flight response) when presented with chemical and visual cues from fish predators (Chiussi et al., 2001). Blue crab post-larvae reduce settlement rates (Welch et al., 1997; Forward et al., 2003), and juveniles initiate escape and alarm responses in the presence of chemical cues from crustacean and fish predators (Diaz et al., 2001). Caridean shrimp (*Tozeuma carolinense*) adjust their microhabitat use when threatened by predators by moving behind seagrass blades to achieve a visual barrier (Main, 1987).

Pacific halibut and Pacific cod are known predators of red king crabs in the field and laboratory (Livingston, 1989; Stoner, 2009); however, halibut exposure impacted crab survival more than cod exposure. Because halibut were located directly on the tank bottom, the direct physical interactions may have been more threatening than those with cod causing more pronounced behavioral responses. Interestingly, behavioral responses scaled with predation risk, as more crabs were eaten by the predator to which they

responded more strongly. The experimental tanks used in the cod trials were larger preventing us from quantifying crab crypsis, thus we cannot make inferences about the effects of cod presence on crab behavior.

Halibut and cod behavior did not vary with crab conditioning. We expected some differences in fish feeding efficiency among conditioning treatments. For example, conditioned crabs had higher crypsis and were presumably more difficult for fish to detect, thus we would expect fewer crabs to be targeted. Juvenile red king crabs increase their dimensions by flaring their limbs and are covered in spines to deter predators. If conditioned crabs had enhanced limb flaring responses, one might expect differences in fish feeding efficiency (i.e., more strikes target^{-1}, fewer crabs eaten target^{-1}, fewer crabs eaten per strike^{-1}). Either the conditioned crabs did not improve their limb flaring postures or fish predators were uninhibited by this defense. Halibut and cod had similar attack rates, but halibut were more efficient predators and are known to consume red king crabs at higher rates compared to Pacific cod in laboratory studies (Stoner, 2009; Stoner et al., 2010). Structural complexity mediates predation of juvenile red king crabs by halibut (Stoner, 2009), thus we expected that crabs deeper within the hydroid mass would experience reduced mortality.

Conditioning potential and implications for stock enhancement

Predation will likely be the first challenge hatchery-cultured red king crabs face once released into the wild. Early benthic phase American and European lobsters are often attacked by fish predators within minutes after release (Wahle and Steneck 1992;

Ball et al., 2001). The loss of juvenile red king crabs to predation must be minimized for release strategies to be feasible. Our study suggests that simple additions of predator exposure to hatchery-rearing protocols prior to release may improve predator avoidance mechanisms and may ease the transition to the natural environment. Behavioral improvements have been noted in other marine species that rely on predator avoidance mechanisms. For example, coho salmon (*Oncorhynchus kisutch*) and summer flounder (*Paralichthys dentatus*) anti-predator behavior can be improved with exposure to predator chemical cues prior to release (Olla and Davis, 1989; Kellison et al., 2000). Queen conch have reduced movement and increased burial behavior with prior predator exposure (Delgado et al., 2002) and faster responses to predators have been noted with abalone (*Haliotis rufescens*) (Schiel and Welden, 1987). European lobsters have faster shelter-seeking behavior with prior experience compared to naïve lobsters (van der Meeren, 2001), and hatchery-cultured blue crabs increase burial rates over time when exposed to natural sediment (Davis et al., 2004). Additional rearing modifications can enhance the quality of juvenile red king crabs. For example, shell coloration can be improved through dietary supplementation (Daly et al., in press), which may enhance visual crypsis.

Before implementing large-scale conditioning programs, it is important to understand the mechanisms of predator exposure. Our results suggest that red king crabs can see and smell halibut held within the same tank but separated by a physical barrier and that direct physical interactions also contribute to predator detection. However, complete predator exposure allows consumption and compromises hatchery production. Because visual and/or chemical cues alone are adequate, a physical barrier or effluent

from separate predator holding tanks will likely be enough to initiate a behavioral response.

Exposure duration may impact the magnitude of the behavioral response. It is encouraging that crypsis improved for naïve and conditioned crabs during the course of the 60 min trials; however, maladapted crabs may have been consumed before behaviorally superior crabs causing the appearance of improved crypsis of individual crabs. While maladapted crabs may have been preferentially consumed, the naive treatment likely had a higher proportion of maladapted crabs. Because we detected higher initial crypsis (t=0 min) and greater survival for conditioned crabs, we suggest that predator exposure improves red king crab behavioral responses. Whether degree of response depends on conditioning duration is a subject for future study.

We demonstrated that hatchery-cultured red king crabs have some degree of behavioral plasticity, which is an important step in refining culturing protocols and developing release strategies for stock enhancement programs. Hatcheries are constantly looking for ways to maximize production; however, behavioral quality is often overlooked. This study shows that red king crab predator avoidance behavior can be improved with conditioning and suggests that hatchery-cultured crabs may adapt quickly to the natural environment. Future studies should investigate alternative conditioning strategies such as exposure to small or benign predators, compare cultured and wild red king crabs to identify potential behavioral or morphological deficiencies that hatchery crabs may acquire through rearing, and investigate benefits of conditioning for *in situ* survival. Short-term exposure to predators could be a cost-effective way to

simultaneously maximize hatchery production and chances of post-release survival for juvenile red king crabs and other crustacean species.

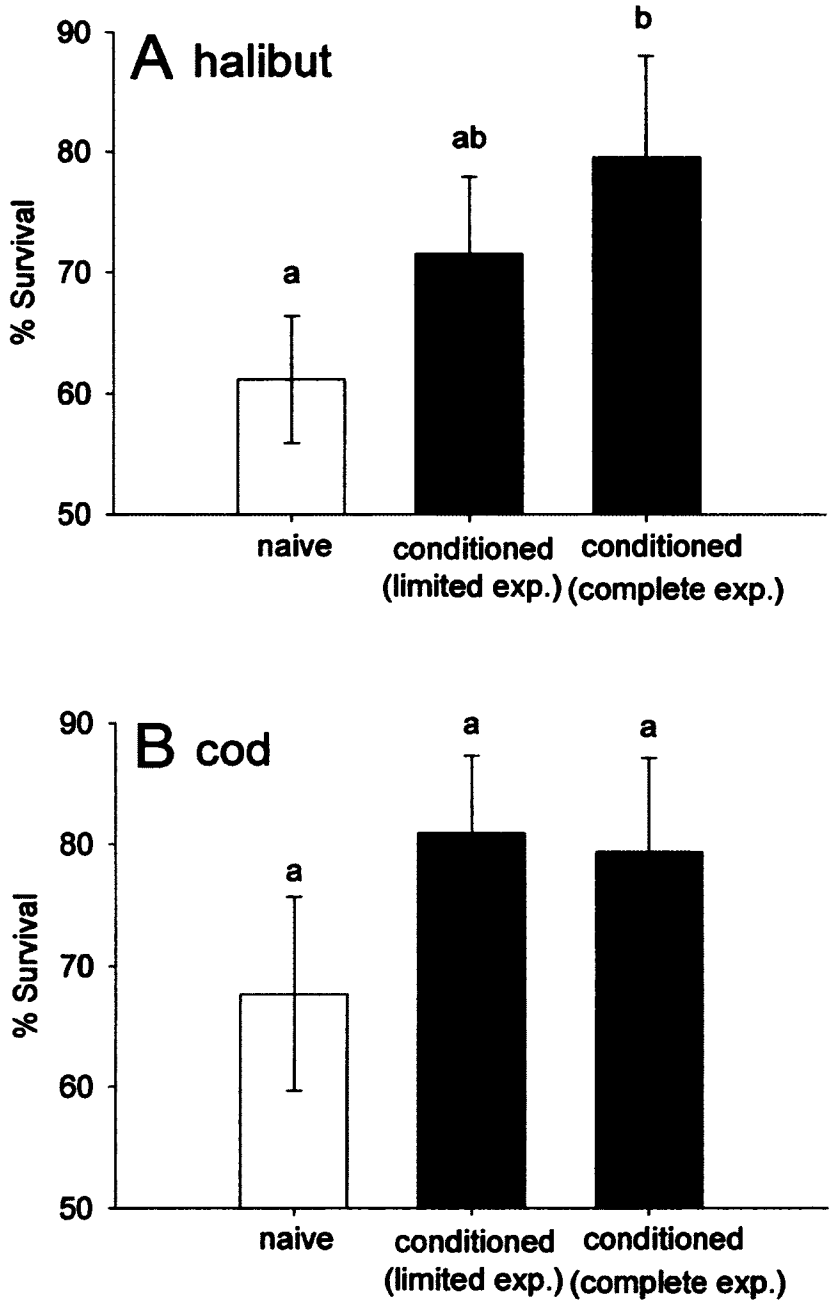

Figure 3.1. Crab survival using (A) Pacific halibut (60 min trials) and (B) Pacific cod (240 min trials) predators. Survival was compared among naïve crabs (white bars), conditioned crabs with limited exposure (visual and chemical cues) (hatched bars), and conditioned crabs with complete exposure (visual, chemical, and physical cues) (grey bars) to predators for 48 h. Values are mean survival ± SE (halibut: n=6, cod: n=9). Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

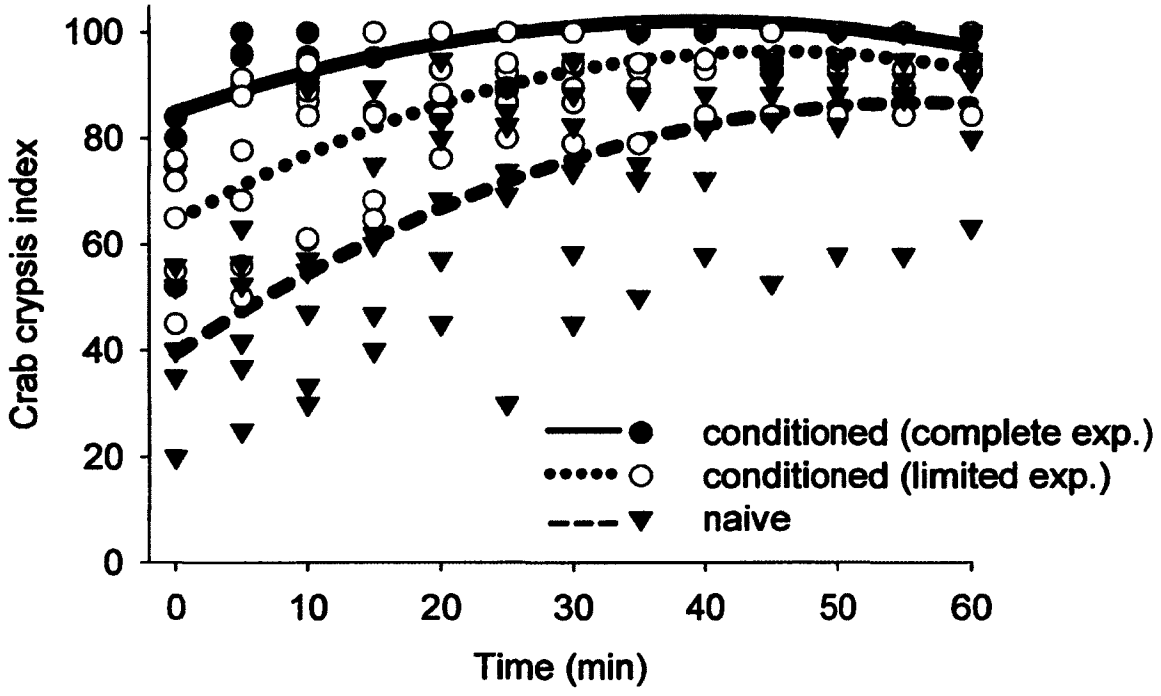

Figure 3.2. Non-linear regression between crab crypsis index and time (60 min halibut trials) for naïve and conditioned crabs with limited (visual and chemical cues) or complete (visual, chemical, and physical cues) halibut exposure for 48 h. Crypsis index is the proportion of crabs not visible (n=6). Not all points are visible because of overlap. Naïve: Crab crypsis index = 39.482 + (1.654 * time) - (0.0145 * time^2), R^2 = 0.491. Conditioned (limited exp.): Crab crypsis index = 64.397 + (1.409 * time) - (0.0155 * time^2), R^2 = 0.542. Conditioned (complete exp.): Crab crypsis index = 84.999 + (0.868 * time) - (0.0110 * time^2), R^2 = 0.583.

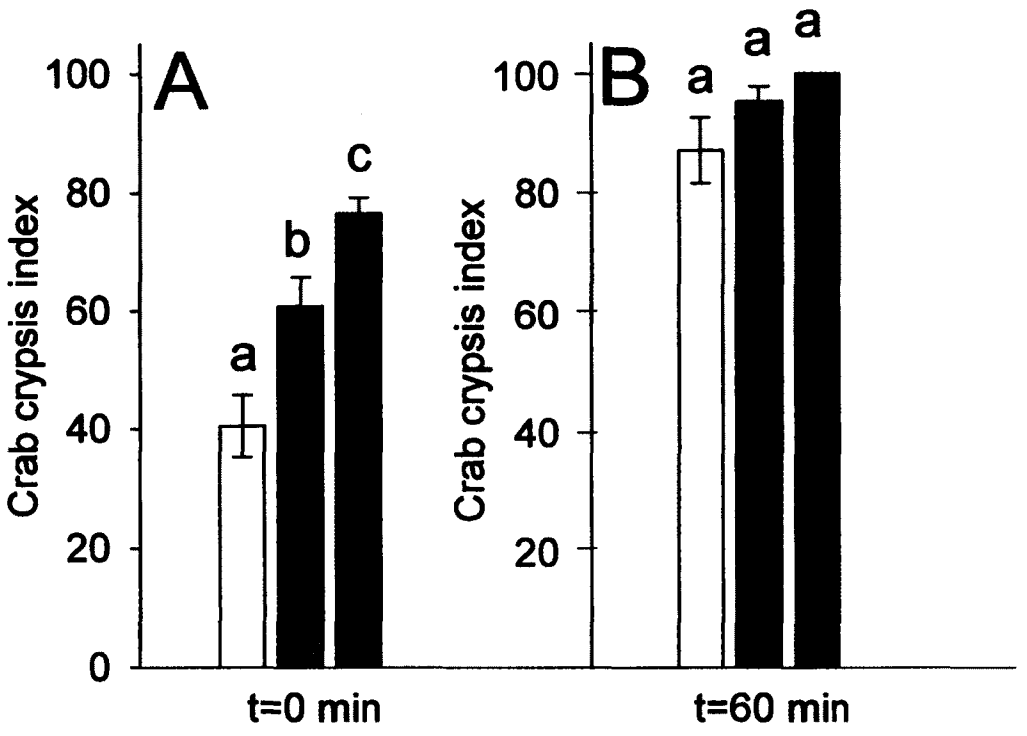

Figure 3.3. Crab crypsis index at (A) time=0 min and (B) time=60 min. Crypsis was compared among naïve crabs (white bars), conditioned crabs with limited exposure to halibut (visual and chemical cues) (hatched bars), and conditioned crabs with complete exposure to halibut (visual, chemical, and physical cues) (grey bars) for 48 h. Crypsis index is the proportion of crabs not visible. Values are mean crypsis index ± SE (n=6). Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

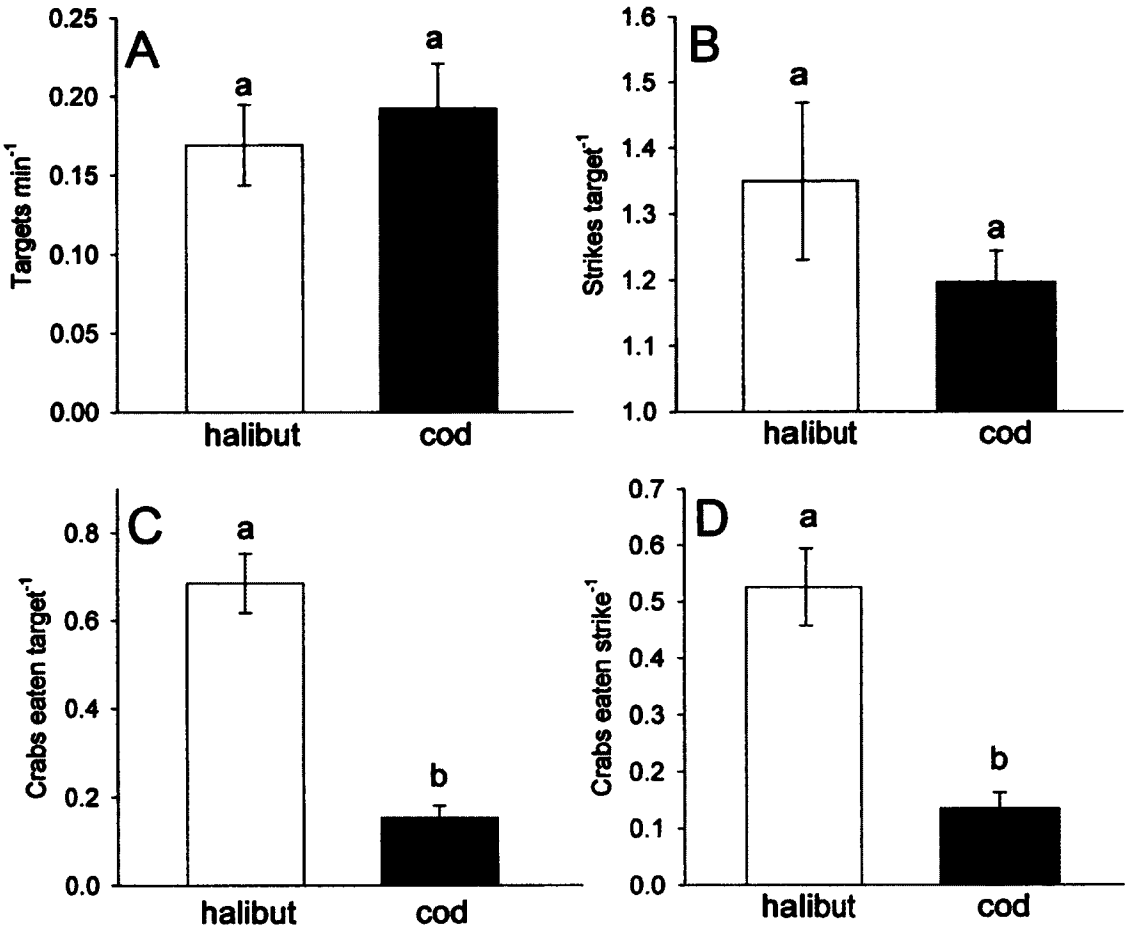

Figure 3.4. Behavioral comparisons between halibut (white bars) and cod (grey bars) for (A) crabs targeted min^{-1}, (B) strikes $target^{-1}$, (C) crabs eaten $target^{-1}$, and (D) crabs eaten per $strike^{-1}$. Values are mean ± SE (halibut: n=6, cod: n=9). Different letters indicate statistical significance (t test, Mann-Whitney, $p \leq 0.05$).

Table 3.1. Parameter estimates for non-linear regressions relating crab crypsis index to time (min) in the halibut predation experiment. Crab crypsis index = A + (B*time) + (C*time^2), where and A, B, and C are parameters.

	Parameters				
	A	B	C	R^2	*p*
Naive	39.482	1.654	-0.0145	0.491	**<0.001**
Conditioned (limited exposure)	64.397	1.409	-0.0155	0.542	**<0.001**
Conditioned (complete exposure)	84.999	0.868	-0.0110	0.583	**<0.001**

Table 3.2. Average ± SE values for fish behavior in halibut and cod predation trials including randomized block ANOVA results. For each behavioral parameter, *df*=2.

		Naïve	Conditioned limited exposure	Conditioned complete exposure	*F*	*p*
halibut	activity index	71.5 ± 6.9	63.9 ± 8.2	62.5 ± 10.8	0.284	0.756
	total crabs targeted	13.0 ± 2.0	9.2 ± 1.9	7.4 ± 3.1	1.304	0.318
	targets min^{-1}	0.22 ± 0.03	0.15 ± 0.03	0.13 ± 0.06	1.736	0.225
	crabs eaten $target^{-1}$	0.68 ± 0.10	0.76 ± 0.10	0.81 ± 0.09	0.373	0.699
	crabs eaten $strike^{-1}$	0.49 ± 0.11	0.59 ± 0.12	0.65 ± 0.11	0.349	0.715
	strikes $target^{-1}$	1.51 ± 0.19	1.45 ± 0.19	1.35 ± 0.15	0.058	0.944
cod	line crossings min^{-1}	13.2 ± 0.8	13.9 ± 1.2	12.1 ± 0.8	1.621	0.246
	total crabs targeted*	49.3 ± 13.0	51.3 ± 13.2	37.3 ± 10.4	0.892	0.440
	targets min^{-1}	0.21 ± 0.05	0.21 ± 0.11	0.16 ± 0.04	0.892	0.440
	crabs eaten $target^{-1}$	0.19 ± 0.06	0.10 ± 0.03	0.17 ± 0.06	4.172	0.048
	crabs eaten $strike^{-1}$	0.18 ± 0.06	0.09 ± 0.03	0.14 ± 0.05	2.539	0.128
	strikes $target^{-1}$	1.18 ± 0.08	1.22 ± 0.06	1.19 ± 0.11	0.062	0.941

* extrapolated from sub-samples

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CHAPTER 4:

Predation Of Hatchery-Cultured Juvenile Red King Crabs (*Paralithodes camtschaticus*) In The Wild[1]

ABSTRACT

The ecologically and commercially important red king crab (*Paralithodes camtschaticus*) is depleted throughout much of the North Pacific and thought to be recruitment limited, making it an appropriate candidate for stock enhancement efforts. Information on predation of newly settled crabs in nearshore habitats is needed to assess the feasibility of large-scale releases. We tethered hatchery-cultured red king crabs of two sizes (range: 1.8-4.0 mm carapace width) in the field for 24 h trials in July and September 2011 and used underwater video cameras to identify predators and predation susceptibility. We identified hermit crabs (*Pagurus spp.*), Alaskan ronquil (*Bathymaster caeruleofasciatus*), Arctic shanny (*Sticheus punctatus*), northern rock sole (*Lepidopsetta polyxystra*), and kelp greenling (*Hexagrammos decagrammus*) as predators. Survival did not vary by body size or deployment month; however, small crabs were consumed sooner than large crabs. Most predation events occurred in daylight hours, with the exception of Alaskan ronquil. Our results suggest stock enhancement efforts should consider predator assemblages when developing release strategies. Future studies should investigate spatial

[1] Daly, B., Eckert, G.L., White, T.D. In review. Predation of hatchery-cultured juvenile red king crabs (*Paralithodes camtschaticus*) in the wild. Prepared for publication in the Canadian Journal of Fisheries and Aquatic Sciences.

variation in predation pressure at multiple locations on broad temporal scales to optimize release strategies and understand population-level effects.

INTRODUCTION

Stock enhancement through the release of cultured juveniles has been used to increase abundance in recruitment limited stocks with varying degrees of success worldwide (Leber et al. 2004; Bell et al. 2006). The foci of previous enhancement efforts were often hatchery production and numbers of individuals released, rather than optimizing post-release survival (Secor et al. 2002; Lorenzen 2005; Stevens 2006*a*). Predation is the greatest ecological hurdle for hatchery-cultured juveniles released in the wild (Bell et al. 2005, 2008; Hines et al. 2008), and intense predation pressure can limit survival of released individuals rendering stock enhancement programs ineffective (Stoner and Davis 1994; Kitada and Kishino 2006). Evaluating predation susceptibility is necessary to assess the feasibility of stock enhancement programs; however, adequate experiments are often not conducted making the effectiveness of many large-scale releases uncertain (Blankenship and Leber 1995; Lorenzen et al. 2010).

Red king crabs (*Paralithodes camtschaticus*) have significant commercial and ecological importance throughout the North Pacific; however, six of the nine stocks in Alaska, USA collapsed in the early 1980s and remain depressed even with decades of no fishing (Orensanz et al. 1998) with only the Bristol Bay and Norton Sound fishing areas consistently open. The Southeast Alaska fishery is intermittently open and closed due to fluctuating estimates of stock abundance (Stratman et al. 2011). Several factors including

overfishing, climate change, groundfish predation, and disease likely contributed to the decline (Orensanz et al. 1998; Stevens 2006*b*; Zheng and Kruse 2006; Bechtol and Kruse 2009). Recruitment limitation has been suggested as a contributing factor for a lack of recovery (Blau 1986) indicating populations may be below carrying capacity, which makes red king crabs a suitable candidate for stock enhancement efforts (Stevens 2006*b*). The Alaska King Crab Research and Rehabilitation and Biology (AKCRRAB) program was created in 2006 to assess the feasibility of stock enhancement for king crabs in Alaska and is the first and only US aquaculture program to successfully demonstrate that king crabs can be cultured on a large-scale in a hatchery setting (Daly et al. 2009). However, hatchery production does not ensure stock enhancement success.

Ecological studies are necessary to develop optimal release strategies for maximizing post-release survival. Factors including sufficient nursery habitat (Kitada and Kishino 2006), size-at-release (Leber 1995; Willis et al. 1995), and release season (Glazer and Jones 1997; Leber et al. 1997; Stoner and Glazer 1998; van der Meeren 2000) impact post-release survival for several fish and invertebrate species. For example, increasing body size reduces predation rates of juvenile American lobsters (*Homarus americanus*) (Wahle and Steneck 1992). Further, survival of hatchery-cultured juvenile blue crabs (*Callinectes sapidus*) increases with body size, but optimal size varies depending on season (Johnson et al. 2008). Critical habitat requirements have been documented for juvenile red king crabs (Loher and Armstrong 2000; Stoner 2009; Pirtle and Stoner 2010; Pirtle et al. in review); however, information on predation of red king crab juveniles in nearshore habitats is scarce, and the importance of body size and

seasonality is unknown for early benthic phase red king crabs, especially age-0 individuals.

Red king crabs have a complex life cycle including four pelagic larval (zoeal) stages, a post-larval (glaucothoe) stage, and benthic juvenile and adult stages (Marukawa 1933). Glaucothoe settle to nearshore nursery habitats and molt to the first juvenile instar where they take the adult-like form (Donaldson et al. 1992; Loher and Armstrong 2000). Juveniles are solitary and cryptic, associating with complex biogenic substrates such as structural invertebrates until approximately age-2 when they begin to display social-aggregative behavior ("podding") and are less cryptic (Powell and Nickerson 1965; Dew 1990, 1991; Stone et al. 1993; Loher and Armstrong 2000). We use the terminology "early benthic phase" to define this solitary, cryptic life history stage (age 0-2), which is ecologically distinct from older juveniles (Wahle and Steneck 1991; Loher and Armstrong 2000).

Predation of juveniles could create a population bottleneck for red king crab. Increases in groundfish abundances coincided with declines in crab populations in Alaskan waters (Zheng and Kruse 2006; Bechtol and Kruse 2009, 2010), yet the role of predation in nursery areas remains uncertain. Groundfish gut content analysis shows that fish eat juvenile red king crabs >50 mm carapace length (CL) (Livingston 1989, 1991; Livingston et al. 1993; Tyler and Kruse 1996), which are approximately 3 years old (Lysenko and Gaidaev 2005), yet most of these observations are outside shallow king crab nursery areas making it difficult to discern population-scale effects of predation on early benthic phase red king crabs that are presumably most vulnerable to predation.

Sculpin (Cottidae), Alaska ronquil (*Bathymaster caeruleofasciatus*), and Pacific halibut (*Hippoglossus stenolepis*) consume age-0 and age-1 red king crab in laboratory and nearshore field experiments (Stoner 2009; Pirtle and Stoner 2010; Pirtle et al. in review; Daly et al. in press a), yet recent evidence shows Pacific cod (*Gadus macrocephalus*) are uninterested in tethered early benthic phase red king crabs in the field (Pirtle et al. in review; this study), which contradicts the dogma that Pacific cod are major predators of early stage red king crabs in the wild (Bechtol and Kruse 2010). Fish predation greatly reduces recruitment of other crab species in Alaska. For example, stomach content analysis off Kodiak Island shows that Pacific cod consumed over 365 million juvenile Tanner crabs (*Chionoecetes bairdi*) (10-45 mm carapace width) in a single bay over 429 days; however, stomach contents did not include red king crabs (Urban 2010). Estimates indicate over 4 billion Tanner crabs (4-34 mm carapace width) were consumed by Pacific cod in the eastern Bering Sea during 1985 (Livingston 1989).

Recent crustacean stock enhancement efforts suggest the importance of predation and post-release survival of hatchery-cultured individuals (e.g., Bannister and Addison 1998; van der Meeren 2000; Ball et al. 2001; Castro et al. 2001; Davis et al. 2005; Zohar et al. 2008). We tethered hatchery-cultured red king crabs in Southeast Alaskan waters to evaluate predation on newly settled early benthic phase crabs with the goal to identify predator species in a nearshore habitat and to assess relative predation pressure during the first juvenile instar stages as part of a king crab stock enhancement feasibility study. We hypothesized that crabs are consumed by a range of predator species, survival rates vary temporally, and that larger crabs have higher survival than smaller crabs.

MATERIALS AND METHODS

Source of juvenile crabs

We cultured juvenile red king crabs as part of AKCRRAB program using established rearing techniques (Daly et al. 2009). Twenty ovigerous females were captured with baited pots in Stephens Passage, Alaska during October 2010. Crabs were transported to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska and placed in 2000 L tanks (2.6 m^2 bottom surface area) containing flow-through ambient seawater and fed 20 g chopped herring and squid per crab twice per week. Once hatching began (spring 2011), larvae from each female were mixed and raised in 1200 L cylindrical tanks until the first juvenile instar stage (C1) in June 2011. Larvae were fed enriched San Francisco Bay strain *Artemia* nauplii daily. *Artemia* nauplii were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media in 100 L cylindrical tanks for 24 h. Juvenile (C1) crabs were shipped to the University of Alaska Fairbanks, Juneau Center, in June 2011 where they were held at a density of ~2000 crabs m^{-2} in 670 L tanks until field experiments began. Holding tanks had flow-through seawater (8°C) and clumps of commercial gillnet for vertical structure. Juvenile crabs were fed Cyclop-eeze® (Argent Chemical Laboratories, WA, USA) and Otohime B1 and B2 (Reed Mariculture, CA, USA) every other day. Crabs exhibited a size range of approximately 1.8-2.5 mm carapace width (CW), stages C1-C2, at 1 month post-settlement (July), and grew to approximately 1.8-4.0 mm CW, stages C1-C4, at 3 months post-settlement (September) (Table 4.1).

Study site

The study site at Yankee Cove (58° 35'431" N, 134° 54'366" W) near Juneau, Alaska (Fig. 4.1) is composed of shallow (0-12 m) nearshore rocky reefs that host dense stands of the kelps *Saccharina subsimplex, Laminaria yezoensis,* and *Agarum clathratum,* several species of prostrate red algae, encrusting algae, and benthic invertebrates (see Pirtle et al. in review). These reefs transition into flat, sandy substrate at approximately 8 m depth. A wide range of vertebrates and invertebrates were observed at the study site and are considered potential predators of early benthic phase red king crabs based on relative size differences. We conducted a series of ten 45 min dives within an area of ~900 m^{-2} at the study site over a one week time span in early July and late August 2011 to document potential predators at the study site. Additionally, we noted the presence of potential predators during video analyses (described below).

Experimental design

We tested the effect of body size, deployment time, and camera light on survival of recently-settled red king crabs during July and September 2011. In each of these two months, we subdivided juvenile red king crabs into two size classes (Table 4.1) representing the upper and lower size range at each time period and compared CW of crabs using t tests. Carapace width was greater for large crabs compared to small crabs in both July (t test, $t=14.369$, $p<0.001$) and September, (t test, $t=87.488$, $p<0.001$). We tethered individual hard-shelled, intermolt crabs by gluing 15 cm of monofilament fishing

line (1.59 kg breaking strength) to the dorsal side of the carapace using a small drop of cyanoacrylate glue (e.g., Heck and Thoman 1981). Tethered crabs were held in the laboratory for approximately 18 h prior to deployment to ensure crabs survived the tethering process and could actively move. Tethered crabs were placed in the field by attaching the monofilament line to a bolt anchored to a concrete disk, which was buried in the substrate and covered with ambient gravel and cobble (~2-30 mm diameter) so that the substrate was flush. Each tethering station was separated by approximately 5 m, and all were at a depth of 8-10 m. On each of 8 consecutive days in both July and September, we deployed 12 crabs in the field for 24 h trials starting at approximately 0900-1000 h. We ensured that crabs were alive and actively moving *in situ* by observing the crabs for several minutes immediately after deployment. In all cases, crabs could move on their tethers, find crevice space within the substrate, and establish crypsis. Of the 12 crabs tethered per day, four (2 small, 2 large) were individually enclosed in separate 2 mm mesh enclosures to prevent predation and escapement during the experiment to assess effects of handling stress (procedural control). Of the remaining eight crabs, four (2 small, 2 large) were tethered without mesh enclosures and with underwater cameras attached to sand anchors and positioned 60 cm above each crab. The remaining four crabs (2 small, 2 large) were tethered without mesh enclosures or cameras. We assessed survival after 24 h and returned crabs remaining at the end of the trials to the laboratory but did not redeploy them. Including the procedural control, a total of 192 crabs were tethered throughout the experiment.

The underwater HD color video cameras (Well-Vu Nature Vision Inc., Manual Wind Color System) (704 x 480 resolution at 7 images sec^{-1}) were used to record predator interactions using methods similar to Pirtle et al. (in review). The cameras had LED lights to improve observations in low light conditions. Lights were adjusted to minimum levels that still allowed for clear observations at night. Cameras were connected to a shore digital video recorder via underwater cable and powered externally by a 12 V battery bank (four marine batteries in series) (see Pirtle et al. in review for details). Trials were excluded from the video analysis if cameras flooded or produced poor image quality. A total of 55 tethered crabs had usable video, of which predators consumed 33 crabs. Though we could not always observe crab behavior in video analysis because of their cryptic nature (hiding in interstitial spaces within the substrate) and small size, some individuals were visible by video. When attacked, the location of crabs was obvious either by fish attack behavior, direct observation of the crab, or by movement of the tether itself. We reviewed videos to identify all species in the field of view as potential predators, actual predators, and quantify time from deployment to the first and mortal attack.

Field and laboratory experiments revealed that predation by sunflower sea stars (*Pycnopodia helianthoides*) is likely an artifact, because untethered juvenile red king crabs can escape by actively moving away (Pirtle et al. in review). Because sunflower sea stars can reach speeds of 12 m h^{-1} (Brewer and Konar 2005) and potentially cover large distances in 24 h, we removed them from the study site prior to crab tethering trials each day. During early July prior to tethering trials, we quantified natural sunflower sea star

density by conducting ten 15 x 2 m random transects in a 30 x 30 m (900 m^{-2}) plot and then removed all sunflower sea stars from the plot over two days. We subsequently quantified sunflower sea star density one, four, five, six and seven days after the removal using ten 15 x 2 m random transects on each day. Densities on each day were compared using repeated measures ANOVA and post-hoc comparisons (Tukey's HSD). We significantly reduced sunflower sea stars (ANOVA, $F=7.81$, $df=5$, $p<0.001$) from average (± SE) initial density of 0.20 ± 0.03 to 0.02 ± 0.01 sunflower sea stars m^{-2} (Tukey's HSD, $p<0.001$) after one day. Sea star densities within the confines of our study site began slowly recovering but remained significantly depressed after 7 days (0.11 ± 0.04 sea stars m^{-2}) (Tukey's HSD, $p=0.044$) (Fig. 4.2), suggesting removal successfully minimized predation by sunflower sea stars. Accordingly, all sunflower sea stars within 10 m of crab tethering locations (eyebolts) were removed immediately prior to each 24 h tethering trial.

Statistical Analysis

We quantified crab survival as percent of deployed crabs remaining after 24 h trials. Percent survival data were arcsine square root transformed and compared among treatments with and without cameras (and camera lights) in July and September using ANOVA and post-hoc comparisons (Tukey's HSD) (Table 4.2). Because the camera effect was not significant ($F=2.94$, $df=1$, $p=0.097$), all replicates with and without cameras were pooled. Survival was then compared among cages (with or without mesh enclosures), sizes (small, large), and deployment months (July, September) using

ANOVA and post-hoc comparisons (Tukey's HSD). Time to mortal attack was compared among small and large crabs using a t test. Differences in time to mortal attack among predators were examined by ANOVA on square root transformed data. All analyses were conducted using Sigma Stat v.4 (Aspire Software International, Ashburn, VA, USA). Statistical significance was set at α=0.05.

RESULTS

We observed many potential predators at the study site (Table 4.3). Diver observations and video analysis suggested that Hermit crabs (*Pagurus spp.*), Pacific cod, sculpins (Cottoidea), northern rock sole (*Lepidopsetta polyxystra*), and kelp greenling (*Hexagrammos decagrammus*) were numerically dominant compared to other species such as Alaskan ronquil, Arctic shanny (*Sticheus punctatus*), and red king crab. Video analysis showed several species passing through the camera field of view but not interacting with the tethered crabs; five species were directly observed consuming crabs (Table 4.3). Hermit crabs were responsible for most (74%) of the mortal attacks on small crabs. Hermit crabs, Alaskan ronquil, Arctic shanny, northern rock sole, and kelp greenling consumed large crabs with relatively similar frequencies (7-36% of total attacks on large crabs) (Fig. 4.3). Video analysis showed that most (81%) of the mortal attacks occurred during daylight hours (Fig. 4.4), but Alaskan ronquil typically attacked at night (2300 to 0400 h). Time to mortal attack differed among predator groups (ANOVA, df=2, F=4.81, p=0.016) (Fig. 4.5), with mortal attacks by Alaskan ronquil occurring later than those by hermit crabs (Tukey's HSD, p=0.012). Arctic shanny and

kelp greenling were excluded from analysis due to low predation frequencies. Juvenile Pacific cod (~15-25 cm total length) were frequently observed in the camera field of view in night time hours feeding on small crustaceans attracted to the camera lights but never interacted with the tethered crabs. Generally, cod activity peaked from midnight until 0200 h.

All crabs in the procedural control survived and remained on their tethers, but survival (average ± SE) for uncaged crabs was 37.5 ± 8.7% for small crabs and 43.8 ± 8.9% for large crabs in July and 21.9 ± 7.4% for small crabs and 34.4 ± 8.5% for large crabs in September (Fig. 4.6). The main effect of mesh cages was significant with uncaged crabs having lower survival than caged (control) crabs (Table 4.4). The main effects of size and deployment month were not significant and there was no size*month interaction (Table 4.4, Fig. 4.6). Time between deployment and mortal attack was longer for large crabs (7.2 ± 1.7 h) compared to small crabs (3.4 ± 1.1 h) (t test, t=-2.306, p=0.028).

DISCUSSION

We show that demersal fishes and crustaceans are predators of small (1.8-4.0 mm CW) juvenile red king crabs in nearshore habitats, which confirm previous red king crab field studies (Jewett and Powell 1979; Pirtle et al. in review). Hermit crabs, Alaskan ronquil, and Arctic shanny consumed both small and large crabs, while northern rock sole and kelp greenling only consumed large crabs. Interestingly, Alaskan ronquil and Arctic shanny were less abundant at the study site than other species that were more abundant

but did not consume tethered crabs. Arctic shanny observed predating tethered crabs were relatively small (~12 cm total length), suggesting early benthic phase red king crabs are not necessarily precluded from small fish predators. Cannibalism is pervasive in red king crab aquaculture (Daly et al. 2009; Stoner et al. 2010), but we did not observe predation by wild conspecifics, likely because natural densities in southeast Alaska (e.g., ~2 crabs m^{-2}) (Loher and Armstrong 2000) are much lower than hatchery conditions (i.e., 2000 crabs m^{-2}). Though we observed juvenile red king crabs (~20-30 mm CW) at the study site during dive surveys, they were relatively rare and were not observed in video analysis, suggesting encounters with tethered conspecifics were uncommon. Further, recently molted crabs are at greatest risk of being predated on due to reduced mobility and lack of defensive armor, thus we tethered hardshell, intermolt crabs, which likely also contributed to our lack of observed cannibalism. We expected predation by sculpins (Cottoidea) (Pirtle et al. in review) and Pacific cod (Bechtol and Kruse 2010), both of which were relatively abundant and appeared in video footage but were uninterested in tethered crabs. Sculpins consume larger tethered red king crabs (4-8 mm carapace length, CL) (Pirtle et al. in review), suggesting they may preferentially target prey items larger than crabs used in our study (1.8-4.0 mm CW). Laboratory studies suggest Pacific cod are not enthusiastic predators of early benthic phase red king crabs compared to halibut (Stoner 2009; Pirtle and Stoner 2010; Daly et al. in press a). Pacific cod consume tethered red king crabs in laboratory studies and do not show negative effects of consuming tethered prey, such as disinterest or choking (Pirtle et al. in review). Our results provide

evidence suggesting Pacific cod are likely relatively little threat to wild, hardshell early benthic phase red king crabs.

Pacific cod predation on larger red king crabs may be significant during molting. Gut content analysis shows that Pacific cod consume large softshell red king crabs (50-160 mm CL) in the eastern Bering Sea (Livingston 1989). Pacific cod may only consume red king crabs when they are in the softshell state and their spines have no anti-predator effect. It is unknown if Pacific cod consume softshell red king crabs in the size range used in our study; however, hardshell Tanner crabs 4-85 mm CW were found in Pacific cod stomachs (Livingston 1989). Further, gut contents from the eastern Bering Sea were only analyzed for Pacific cod over 30 cm fork length; however, Pacific cod 17-22 cm fork length consumed hardshell red king crabs 5.0-7.5 mm CL in laboratory studies (Pirtle et al. in review; Daly et al. in press a) suggesting our lack of observed Pacific cod predation on tethered red king crabs was not necessarily related to the relatively small Pacific cod body size (~15-25 cm) or hardshell state of the crabs. As such, we cannot discount the potential importance of Pacific cod predation on early benthic phase red king crabs in nearshore habitats especially during molting, but suggest a lack of interest of Pacific cod on early benthic phase red king crabs.

The prevalence of hermit crab predation was unexpected. We assessed the possibility of a tethering artifact by placing an untethered red king crab and a hermit crab (of typical size observed in video analysis) in a laboratory tank to determine if juvenile red king crabs could escape hermit crab predation. In each of three trials, different hermit crabs easily captured and consumed untethered red king crabs, suggesting hermit crab

predation was not necessarily a tethering artifact. Hermit crabs observed in video analysis were large (up to approximately 140 mm shell length) and capable of short bursts of speed, which enhances their ability to capture and consume wild juvenile red king crabs. Hermit crabs are also known predators in other systems (Pechenik et al. 2010). Therefore, given their abundance and our results, hermit crabs may be significant consumers of recently-settled red king crabs in nearshore habitats.

Almost all predation occurred in daylight hours. Interestingly, predation by Alaskan ronquil occurred exclusively during hours of darkness, despite appearing in video footage in daylight hours, which confirms previous observations (Pirtle et al. in review). It is unclear from video footage why Alaskan ronquil predated exclusively at night, though camera light likely enabled visual detection. Tethered juvenile spiny lobsters (*Jasus edwardsii*) are preyed on by fish during daylight hours and invertebrates (crabs, octopus) during the night (Mills et al. 2008). As such, the importance of visual detection and foraging activity likely varies with predator species (Aksnes and Giske 1993). Predation was not observed during the dawn or dusk phases, suggesting these time spans may be optimal for releases. Further, dusk releases may provide crabs additional time to establish crypsis prior to daylight hours where predation by fish predators can be most intense.

Survival did not vary among the size ranges of animals used in this study (1.8-4.0 mm CW). We expected to find improved survival with increasing body size, as observed for other crustacean species including American lobsters (Wahle 1992; Wahle and Steneck 1992), spiny lobsters (*Panulirus argus*) (Smith and Herrnkind 1992), and blue

crabs (Hines and Ruiz 1995; Johnson et al. 2008); however, these differences in predation vulnerability often correspond with broader size ranges than we used in our study. For example, Wahle and Steneck (1992) demonstrate American lobster size specific survival among 5-7, 15-20, and 30-40 mm CL size classes. Yet not all studies find improved survival with increasing size. Pirtle et al. (in review) failed to detect size specific survival between tethered age-0 (4-8 mm CL) and age-1 (16-28 mm CL) red king crabs at the same study site, though sample sizes were small. Further, Pirtle et al. (in review) found that all age-1 crabs tethered in gravel and shell hash were consumed by sunflower sea stars, making survival rate comparisons to the present study difficult as we removed sunflower sea stars from the study site to avoid tethering artifacts. Artificially reared winter flounder (*Pleuronectes americanus*) do not differ in predation risk with slight increments in size or age in laboratory experiments (Bertram and Leggett 1994). In our study, large crabs took twice as long to be consumed as small crabs; however, behavioral differences among size classes (e.g., limb flaring, aggressive postures) were not apparent in video analysis. We suggest any differences in predation risk among red king crabs within the size range in our experiment (1.8-4.0 mm CW) are subtle and potentially ecologically inconsequential.

It is generally understood that seasonal timing of release of hatchery-cultured individuals impacts survival either by effects of size-at-release, water temperature, food availability, or related shifts in predator suites (Bertness et al. 1981; Leber et al. 1997; Stoner and Glazer 1998; van der Meeren 2000; Johnson et al. 2008). For example, European lobster (*Homarus gammarus*) and queen conch (*Strombus gigas*) survival is

lowest in summer compared to spring and fall months, due to higher predator densities in the summer (Stoner and Glazer 1998; van der Meeren 2000). We did not detect temporal variation in survival, likely because of minimal predator assemblage fluctuations associated with the relatively short time span between trials.

Extended hatchery rearing may not be beneficial if relative survival rates do not vary temporally or improve with body size. Cannibalism in the hatchery causes high mortality rates (e.g., 1.8% mortality d^{-1}) at typical rearing densities (e.g., 2000 crabs m^{-2}) (Daly et al. in press b). Early post-settlement (C1 or C2 instar stage) release eliminates opportunity for cannibalistic mortality associated with high density hatchery rearing conditions. Individuals released in the wild could disperse and potentially establish crypsis, which could minimize density dependent cannibalism and potentially allow for higher survival rates compared to hatchery conditions. Thus, releasing crabs soon after settlement may beneficial for stock enhancement efforts.

We acknowledge that the tethers and camera light create artifacts (i.e., enhanced predation) (Zimmer-Faust et al. 1994), and that our results cannot be used to measure absolute predation rates. However, these studies are useful to identify predators and to evaluate relative predation rates. Camera lights could have impacted predator behavior or species composition; however, crab survival was similar with and without the use of cameras, indicating that the lighting did not result in increased predation.

Tethered red king crabs showed no obvious behavioral deficiencies (e.g., prolonged immobility, abnormally high activity) that may exacerbate predation. For example, hatchery-cultured European lobsters are highly susceptible to fish predation

immediately after release because of abnormal swimming behavior or prolonged immobility induced by stress associated with transportation to the release site (van der Meeren 1991). The tethered red king crabs typically found crevice space soon after deployment and were highly cryptic. Substrate with adequate crevice space is likely important for survival of early benthic phase red king crabs by reducing susceptibility to predators, especially immediately after deployment. Similarly, artificially reared early benthic phase European lobsters require cobble and gravel substrate to reduce *in situ* predation by small benthic fishes (Ball et al. 2001).

Our study lays groundwork for developing red king crab release strategies. We show that hatchery-cultured red king crabs can survive in the wild, at least for 24 h, and suggest that differences in predation susceptibility may be ecologically inconsequential for post-release survival during the first juvenile stages. Early benthic phase red king crabs are consumed by a range of fishes and invertebrates including hermit crabs, Alaskan ronquil, Arctic shanny, northern rock sole, and kelp greenling, thus predator assemblages and the timing of predation should be considered when selecting release sites and time-of-release. Future studies should examine spatial variation in predation pressure at multiple locations on broad temporal scales to understand population-level effects. Benefits of larger size increments associated with extended (i.e., >1 year) hatchery grow-out and benefits of *in situ* acclimation or hatchery conditioning should also be explored. Further, a fundamental understanding of mechanisms causing low population abundances is necessary for stock enhancement to succeed. For example, additions of juveniles will likely be unsuccessful if recruitment limitation is caused by

predation mortality; however, if recruitment limitation is caused by an inadequate supply of settling larvae from depressed spawning stocks or low reproductive success, stock enhancement could show promise.

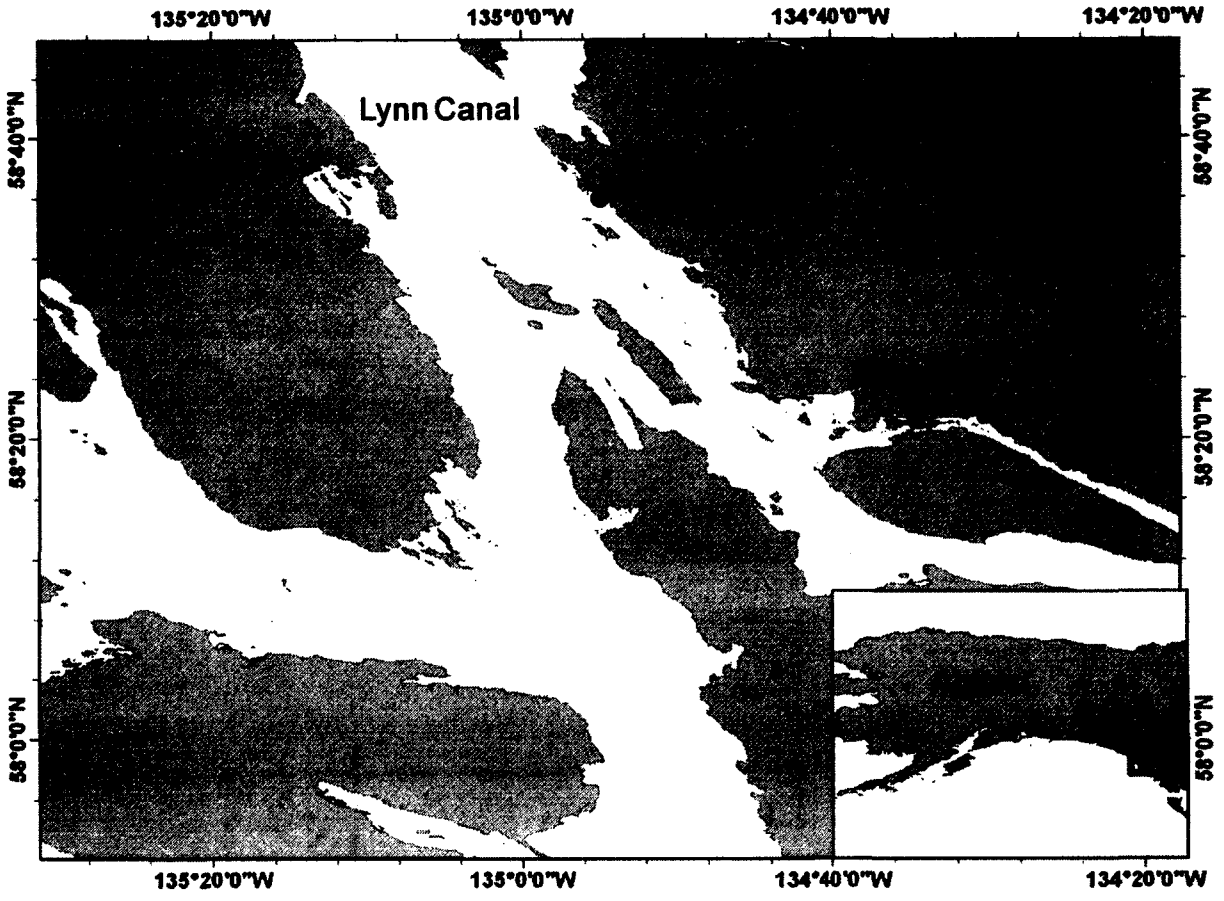

Figure 4.1. Map of southern Lynn Canal, Alaska showing the study site location.

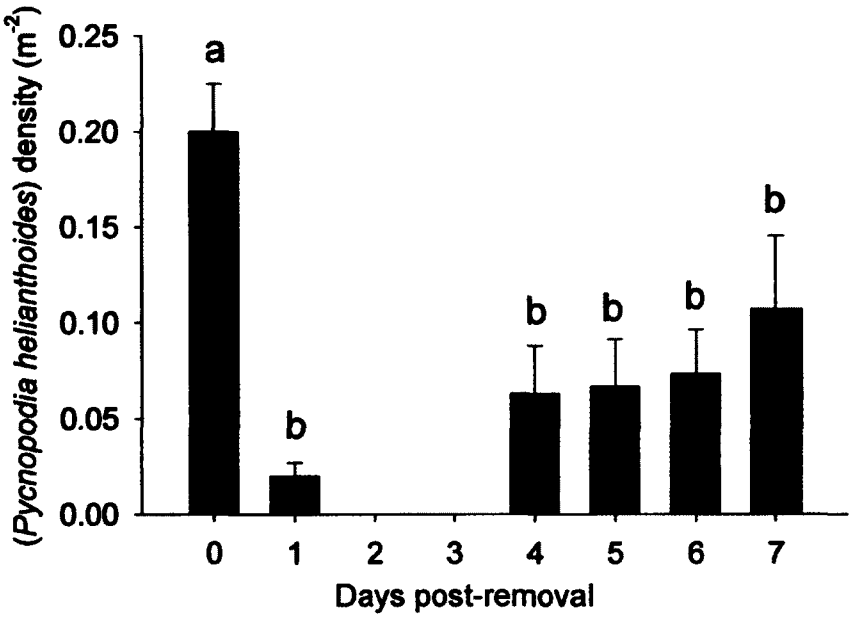

Figure 4.2. Average ± SE sunflower sea star (*Pycnopodia helianthoides*) density over time. Densities were lower on each day after the initial removal (day 0) (Repeated measures ANOVA, $F=7.81$, $df=5$, $p<0.001$). Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

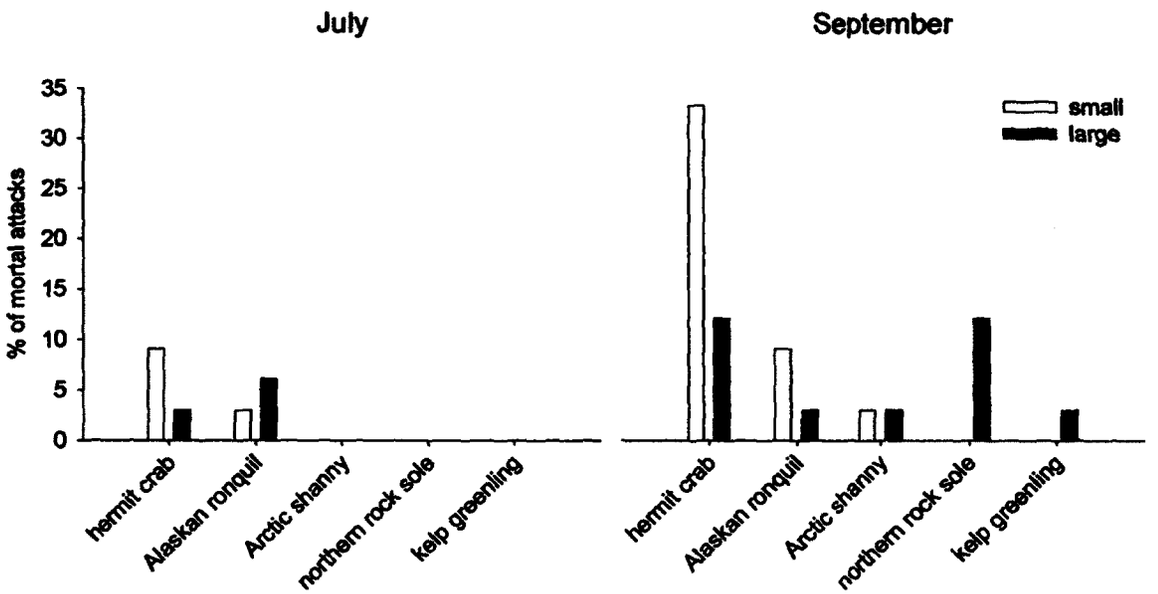

Figure 4.3. Percent of total mortal attacks observed in video footage by predators on small and large crabs in July and September. n=33 crabs consumed of 55 tethered with cameras. These data represent 16 separate 24 h trials (8 in July, 8 in September).

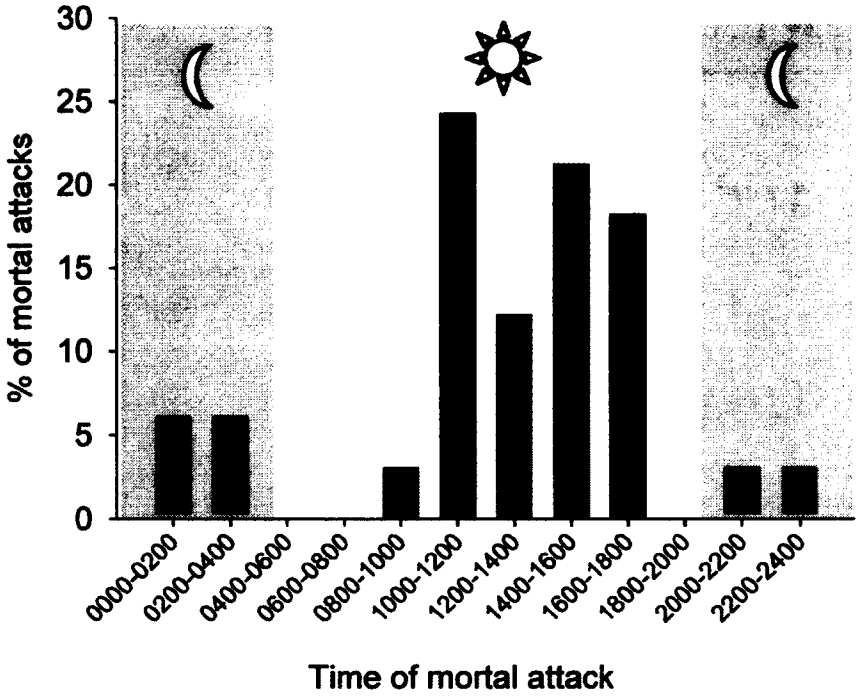

Figure 4.4. Percent of total mortal attacks observed in video footage by time of day over the 24 h trials. n=33 crabs consumed of 55 tethered with cameras. These data represent 16 separate 24 h trials (8 in July, 8 in September). Crabs were deployed at approximately 0900-1000 h for each trial.

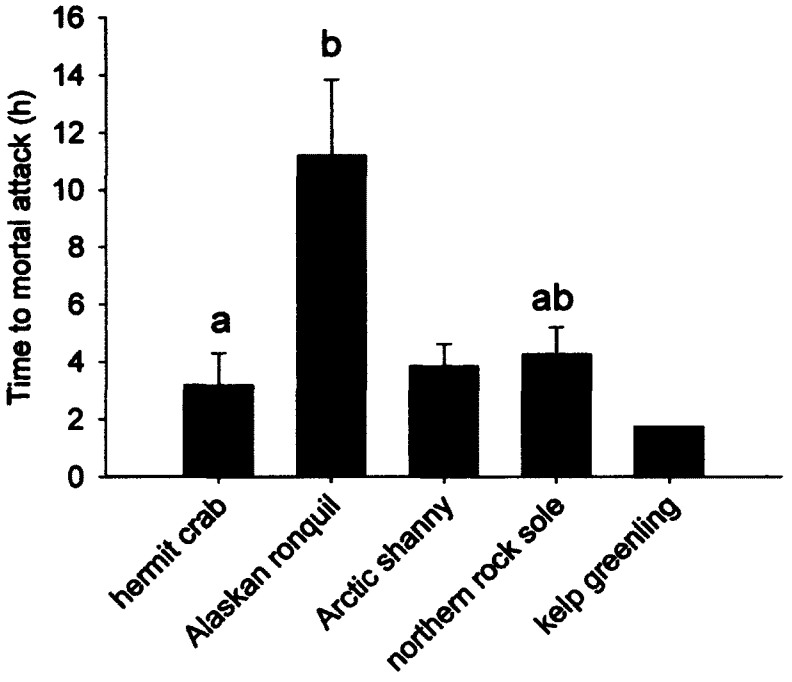

Figure 4.5. Average ± SE time between when crabs were deployed and when they were consumed by predators. Time to mortal attack differed among predators (ANOVA, df=2, F=4.81, p=0.016). Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$). Arctic shanny and kelp greenling were excluded from analysis because of low predation frequencies.

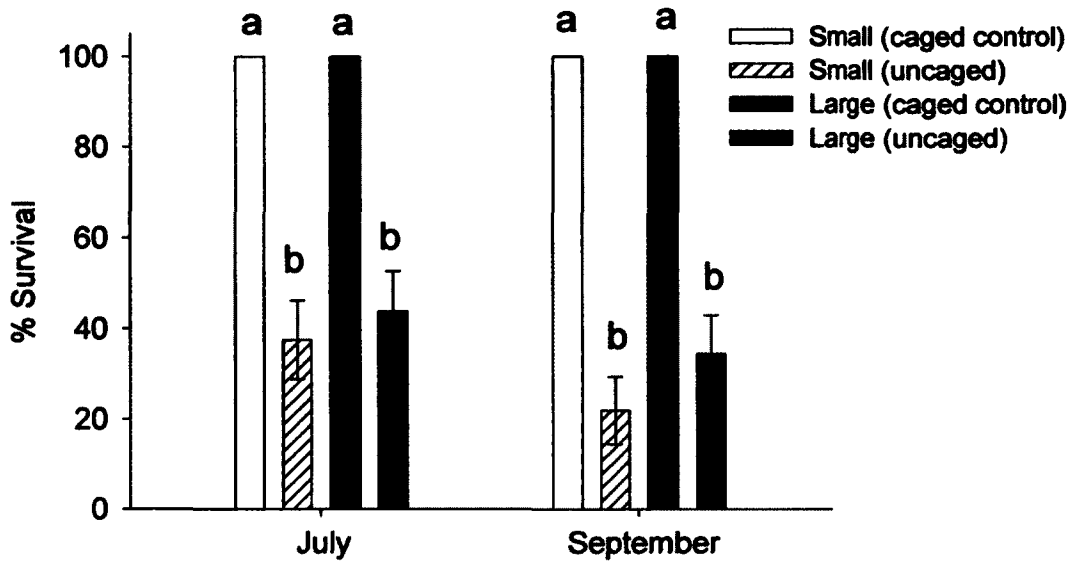

Figure 4.6. Average ± SE survival in July and September for small and large crabs. n=8 for all treatments. Different letters indicate statistical significance (Tukey's HSD, $p \leq 0.05$).

Table 4.1. Average ± SE carapace width and estimated juvenile instar stage for "small" and "large" crabs used in July and September tethering trials.

Month	Size class	Carapace width (mm)	SE	Instar stage
July	Small	1.90	0.014	C1
	Large	2.40	0.027	C2
September	Small	2.07	0.012	C1, C2
	Large	3.86	0.017	C4

Table 4.2. ANOVA for survival of red king crab (*P. camtschaticus*) juveniles deployed with and without cameras in July and September.

Effect	SS	*df*	MS	F	*p*
Camera	0.277	1	0.277	2.94	0.097
Month	0.099	1	0.099	1.05	0.315
Camera x month	0.001	1	0.001	0.01	0.926
Residual	2.639	28	0.094		

Table 4.3. Taxa present at the study site that were considered potential predators of recently-settled red king crabs. Taxa that were observed during dive surveys (Occur), appeared in the camera field of view (Appear), and successfully consumed a tethered crab (Consume) in video analysis are indicated with an "X". Relative abundances are indicated as "low" (L), "moderate" (M), or "high" (H). We removed sunflower sea stars from the study site resulting in no predation by sunflower sea stars on tethered crabs.

Common name	Species name	Occur	Appear	Consume	Abundance
Pacific cod	*Gadus macrocephalus*	X	X	-	H
Walleye pollock	*Theragra chalcogramma*	X	-	-	L
Kelp greenling	*Hexagrammos decagrammus*	X	X	X	H
Whitespotted greenling	*Hexagrammos stelleri*	X	X	-	L
Undefined rockfish	*Sebastes spp.*	X	-	-	M
Buffalo sculpin	*Enophrys bison*	X	-	-	M
Great sculpin	*Myoxocephalus polyacanthocephalus*	X	X	-	H
Crested sculpin	*Blepsias bilobus*	X	-	-	L
Silverspotted sculpin	*Blepsias cirrhosus*	X	-	-	M
Red Irish lord	*Hemilepidotus hemilepidotus*	X	X	-	H
Undefined sculpins	*Artedius, Clinocottus, or Oligocottus spp.*	X	X	-	M
Sturgeon poacher	*Agonus acipenserinus*	X	X	-	H
Arctic shanny	*Sticheus punctatus*	X	X	X	M
Northern ronquil	*Ronquilus jordani*	X	X	-	M
Alaskan ronquil	*Bathymaster caeruleofasciatus*	X	X	X	M
Starry flounder	*Platichthys stellatus*	X	-	-	M
Yellowfin sole	*Limada aspera*	X	-	-	M
Northern rock sole	*Lepidopsetta polyxystra*	X	X	X	H
Crescent gunnel	*Pholis leata*	X	X	-	M
Giant Pacific octopus	*Enteroctopus dofleini*	X	-	-	L
Red king crab	*Paralithodes camtschaticus*	X	-	-	L
Graceful kelp crab	*Pugettia gracilis*	X	-	-	L
Undefined hermit crabs	*Pagurus/Elassochirus spp.*	X	X	X	H
Undefined shrimps	*Pandalidae*	X	-	-	L
Tubesnout	*Aulorhynchus flavidus*	X	-	-	M
Wolf eel	*Anarrhichthys ocellatus*	X	-	-	L
Sunflower sea star	*Pycnopodia helianthoides*	X	X	-	H[1], L[2]

[1] Abundance before removal

[2] Abundance after removal

Table 4.4. ANOVA for survival of red king crab (*P. camtschaticus*) juveniles deployed with and without mesh enclosures (cage effect), of two size classes (small, large), and in two months (July, September). Bold indicates statistical significance ($\alpha \leq 0.05$).

Effect	SS	*df*	MS	F	*p*
Cage	15.642	1	15.642	241.67	**<0.001**
Size	0.047	1	0.047	0.72	0.399
Month	0.133	1	0.133	2.05	0.157
Size x month	0.002	1	0.002	0.03	0.859
Residual	3.625	56	0.065		

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GENERAL CONCLUSIONS

The integration of aquaculture with capture fisheries will likely be needed to help meet projected increases in future global seafood demand. Many past stock enhancement efforts have failed because of an inability to quantify effects of released individuals on wild populations, lack of integrated management, and prioritizing hatchery production over ecological considerations; however, I suggest that much can be learned from the mistakes of past enhancement programs, especially with increasing advances in aquaculture technology, genetics, tagging, and fishery modeling. My research provides an important step for developing a responsible red king crab stock enhancement program by addressing hatchery bottlenecks and methods for optimizing chances for post-release survival. Though we now have a better understanding of hatchery rearing from larval hatching to the early benthic phase, cannibalism during the juvenile stages is an ongoing challenge. I illustrate specific improvements in rearing technology that mediate cannibalism and improve the economic feasibility of hatchery production.

The largest unknown for the success of a red king crab stock enhancement programs is survival after release. Once deployed in the wild, crabs must adapt to their new surroundings, find shelter, and avoid predation. Structural complexity is an important habitat requirement for establishing crypsis; however, crabs must be behaviorally competent to effectively utilize structure for protection from predators. Further, release timing and location will likely influence survival for at least several molts and must be considered for optimizing release strategies. I demonstrate that hatchery-cultured red king crabs are morphologically and behaviorally plastic, which

suggests conditioning in captivity prior to release may improve ability for crypsis in the wild. I lay the groundwork for developing release strategies by identifying predator species in a nearshore habitat, assessing relative predation pressure during the first juvenile instar stages, and demonstrating that hatchery-cultured red king crabs have no obvious behavioral deficiencies and can survive for 24 h in the wild.

Though there has been a considerable expansion of knowledge about early life history red king crabs in recent years, future research is needed to understand if wild populations can be enhanced in Alaska through large-scale releases of cultured juveniles. A comprehensive assessment of temporal and spatial variation in predation pressure, potential release sites, and field experiments to quantify the fitness of hatchery-reared compared to wild individuals and survival of released individuals over an extended period are key initial steps in assessing the potential of restocking. Further, hatchery technologies must be advanced for large-scale production to fuel enhancement and produce juveniles at the size and time at which they are most likely to survive in the wild. As bottlenecks in hatchery production and survival of released juveniles are overcome, stock enhancement will become increasingly feasible for red king crabs in Alaska.